

Phenotypic evaluation of floral characteristics for predicting the components of longer floral retention in *Hibiscus rosa sinensis* L. hybrids

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Explicit determinants of flower retention are necessary in the breeding and selection including the study of these processes. As an example, *Hibiscus rosa-sinensis*, a widely accepted ornamental crop for its aesthetic value, has ephemeral floral retention, thus it cannot be marketed as a cutflower. This limits the potential of this plant for marketing only as potted or hedge plant. To overcome this constraint, studies are necessary to determine the factors which may contribute to extend the floral retention of *Hibiscus*. Moreover, the effects of morphology on the progression of abscission is not closely studied. In view of this problem, this research was conducted primarily to evaluate the phenotypic traits and predict the morphological characters that may be used to identify breeds with longer retention on the plant. Four breeds were used, namely: Reddy-or-Not (RON) and Wilcox (WX) representing one-day old variety, while Loren Legarda x Estrella F. Alabastro (LLxEFA) and Gelia Castillo (GC) represented two-day old varieties. Floral retention on the plant was quantified from each breed and found to be differential with 13 hr for RON, 14 hr for WX, 47 hr for LLxEFA and 51 hr for GC. Morphology of the flowers was studied using four characters: peduncle diameter at attachment site to the receptacle, peduncle length, peduncle diameter and petal thickness at the attachment site. All characters except peduncle length correlated very strongly with floral retention. Characters that are very strongly correlated with floral retention were selected and

subjected to linear regression analysis to generate predictive values. For the regression analyses, the following are the characters and their model's respective level of adequacy or determination includes: the uppermost diameter ($R^2=85\%$); the receptacle diameter ($R^2=92\%$); and the petal thickness at the attachment site ($R^2=71\%$). It is recommended to use the receptacle diameter as the primary determinant of flower retention in a breed, since it is conspicuous and, at the same time, this had the highest level of adequacy or determination of 92%. Using the predictive models generated, this will allow the efficient selection and evaluation process to identify the best breed/s despite of the high number of samples being evaluated in the *Hibiscus* breeding program.

KEYWORDS

abscission, *Hibiscus rosa-sinensis*, morpho-anatomy, prediction, retention

INTRODUCTION

The length of flower retention is a vital characteristic in plants. Economically, floricultural crops are much more appreciated if they exhibit flowers at a longer time on the plant. However, ephemeral flowers like the hibiscus cannot be used as a cutflower because it is short-lived. In addition, this is essential biologically because at this stage, reproduction commenced, but a failure of this stage could result to a compromised productivity in any of the flowering plants. The flowering stage allows the plant to reproduce, sustain their lineage, and finally produce offspring. Flowering, to be successful, is dependent on several

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factors and the key to which is the longevity of the flowers. With greater floral longevity, the flower would have a greater chance of success of being pollinated (Rathcke 2003). Moreover, control of flowering follows the control of the successive stages of plant reproduction, hence a need to investigate the longevity of flowers on the plant.

Hibiscus rosa-sinensis L. possesses favorable characteristics that are necessary to study floral retention. The reasons for this are the following: i) *Hibiscus* has ephemeral flowers that usually last for only one day (Gillman 1999, Woodson et al. 1985, Magdalita 2011), ii) The stages of flower retention on the plant could be easily observed since the big flowers are explicitly displayed on the plant, iii) Flowers exhibit inter-breed variation in terms of floral longevity (Magdalita 2011), and iv) *Hibiscus* is an economically important ornamental 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 in the Philippines (Nakasone and Rauch 1980, Australian *Hibiscus* Society 2007, Kuligowska et al. 2013, Magdalita and Pimentel 2013, Magdalita et al. 2016). The Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS) at the 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 (Pascual and Magdalita 2012, Dolores et al. 2014, Dolores et al. 2016). This institution has been producing many varieties since 1995 which are collectively called the UPLB *Hibiscus* hybrids falling under the different series like the 'Centennial', 'Millenium', 'Celebrity Star', 'Oblation', 'Women in Public Service', 'Women in Science', 'Women Saints and Institutions Named after Them' and 'Women in Social Entrepreneurship'. To date, there were already 46 hybrid varieties that were released (Pimentel 1999, Magdalita et al. 2009, Magdalita and Pimentel 2010, Magdalita et al. 2011, Magdalita and Pimentel 2013, Magdalita et al. 2016).

However, the flower of these UPLB *Hibiscus* hybrids normally lasts for one to two days only. This constraint limits the use of *Hibiscus* as a cutflower for floral arrangements and decorations. While two-day old new *Hibiscus* hybrids have been developed, they should be further hybridized with other *Hibiscus* species to generate hybrids with longer floral retention in order for *Hibiscus* to rival other cutflowers. Before this could be achieved, the factors and mechanisms affecting retention of the flowers on the plant need to be investigated, and predictive phenotypic traits for longer floral retention on the plant should be established. Hence, the aims of this study were to: i) assess the floral morphology to elucidate characters that affect or determine floral retention; ii) quantify the duration of floral retention across the breeds used; and iii) correlate and regress through statistical methods to establish prediction equations. This study was conducted at the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines from April 2014 to May 2015.

MATERIALS AND METHODS

Plant materials

Plant materials were grown under field conditions at the Institute of Plant Breeding Complex, College, Laguna, Philippines. The breeds used were: "Reddy-or-Not" (RON) and "Wilcox" (WX)

representing one-day old varieties and, the hybrids "Loren Legarda x Estrella F. Alabastro" (LLxEFA), and "Gelia Castillo" (GC) representing two-day old varieties (Figure 1). All varieties were generated from the *Hibiscus* Breeding Program at the IPB under the leadership of the principal breeder, Dr. Pablito M. Magdalita. The length of floral retention in terms of the number of hours was quantified from full flower opening early in the morning or anthesis until the petals abscised in the late afternoon. Thirty flower samples were collected at different times of the year as soon as the plant materials bloomed.



Figure 1: Breeds of *Hibiscus rosa-sinensis* L. used in the study: a) 'Wilcox' (WX); b) 'Reddy-or-Not' (RON); c) an unreleased hybrid variety, 'Loren B. Legarda' x 'Estrella F. Alabastro' (LLxEFA); and d) 'Gelia Castillo' (GC).

Assessment of quantitative\ morphological traits of selected floral parts

Morphological indices relative to the length of floral retention, were established. This involved assessment of several floral characters namely: peduncle diameter at the attachment site to the receptacle, peduncle length and petal thickness at the attachment site. Separate measurements involving thirty (30) flower samples for each breed were used for measuring each character. The peduncle length and diameter including receptacle diameter were measured using a Vernier caliper. Petal thickness was measured at the attachment site using a Micrometer caliper.

Statistical Design and Analyses

Data analyses were done using Microsoft Excel 2010 and Statistical Tool for Agricultural Research 1.1 (STAR) (IRRI 2014). In studying the floral morphology, 30 flower samples were utilized per character in each breed.

The experiment was laid out in Completely Randomized Design (CRD) with three replications. Pearson's correlation analysis was employed to find out the association of floral retentions with morphological measurements. All data were analyzed using one-way ANOVA using the F-test. Significant differences between treatment means were subjected to Pair-Wise Mean Comparison using the Least Significant Difference (LSD). In establishing predictive equations, Linear Regression was used on the characters that gave positive trend indicating very strong relationship of morphological traits with floral retention length.

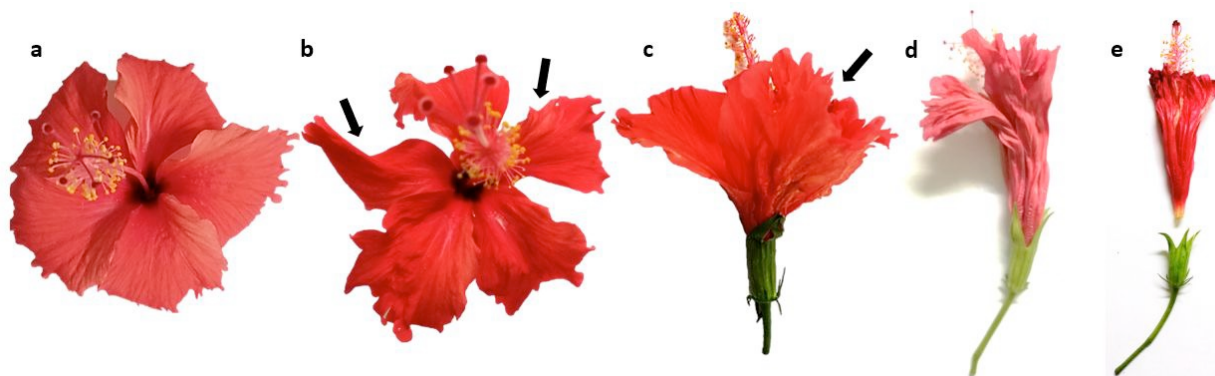


Figure 2: Flower stages of *Hibiscus* flowers starting from a) flower opening that persists for time until b) full bloom, followed by c) petal folding (on white arrows) that indicates d) closure after a few hours until e) final abscission.

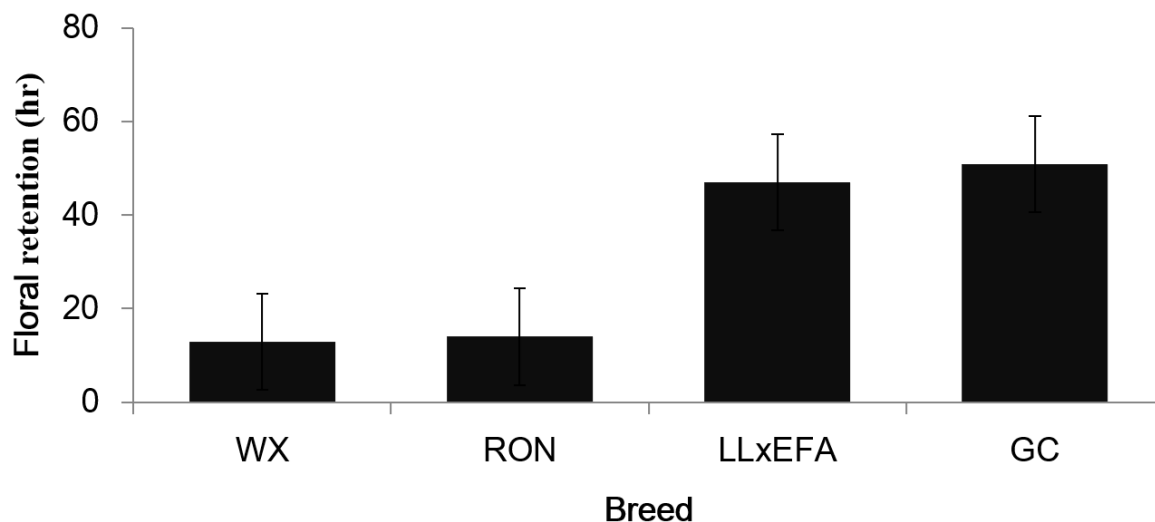


Figure 3: Duration of floral retention (hr) across breeds measured from full opening to petal abscission.

RESULTS AND DISCUSSION

Varying duration of petal retention across breeds

Floral stages in *Hibiscus* are characterized by major phases such as i) flower opening that persists for time until ii) full bloom followed by iii) petal folding which indicates iv) closure after a few hours until v) final abscission (Figure 2). First, the flower bud opens into its full bloom. Flowers remain opened after hours and floral closure is signified by petal folding or twirling. The petals continually fold until the full closure of the flower. At this stage, the flowers will stay a few more hours before fully abscising from the other floral parts.

The varying duration of petal retention from full opening until flower abscission across the four breeds was measured (Figure 3). In WX, petals stayed 13 hr starting from full bloom up to full abscission (Figure 2), while in RON, petals stayed for 14 hr. These two breeds were previously identified as 'one-day' old breeds. In contrast, LLxEFA flowers stayed for 47 hr suggesting that the petals abscised only after two days (Figure 3). The same observation is true for GC wherein the petals stayed attached in the flower for 51 hr. The observations in the number of hours of petal retention in the flower also distinguished the breeds from each other. GC had the longest duration of floral retention; LLxEFA was next to it, followed by RON and then WX which had the shortest duration of petal retention.

Hibiscus rosa-sinensis petals are usually retained in the flower for one day only (Purseglove 1968). However, with breeding, there were new varieties produced which could endure longer than the typical one-day old breeds. In line with this, this study attempted to find out how long breeds such as WX, RON,

LLxEFA, and GC will retain their flowers on the plant. These four breeds opened, closed and abscised at different times of the day.

This species-level of variability in duration of floral retention allows the use of this plant as a model species for studying variation in floral retention to infer how abscission progresses inter-specifically. This would allow us to understand further how retention varies from one species to the other and elucidate the mechanisms necessary to lengthen floral retention of ephemeral flowers like the hibiscus.

Most probably, the *H. rosa-sinensis* breeds showed variability in their floral retention length to have a greater chance of being pollinated and to be admired better by the on-lookers. According to Marques and Draper (2012), the risk that flowers would not be pollinated is one of the problems that plants with ephemeral flowers should overcome. They further asserted that floral longevity is fundamental to plant reproductive fitness. This guideline on the viability of flowers as a determinant of their own chance of pollination is natural and has been proven experimentally (Rathcke 2003, Giblin 2005, Abdala-Roberts et al. 2007). Plants need greater chance of pollination even though this process may be difficult to enable them to ensure their perpetuation (Rathcke 2003, Giblin 2005, Abdala-Roberts et al. 2007).

Morphology and floral retention

In this study, parts of the *Hibiscus* flowers that were surveyed were shown in Figure 4. Petal thickness, receptacle diameter, peduncle diameters and length were studied. Also, shown in Figure 4 are the abscission zones in a *Hibiscus* flower. In these

areas, several parts disengage with each other, usually in sequence: first, the corolla separates from the receptacle; secondly, the receptacle and the calyx separate from the peduncle; and thirdly, the peduncle separates from the plant where the flower attaches.

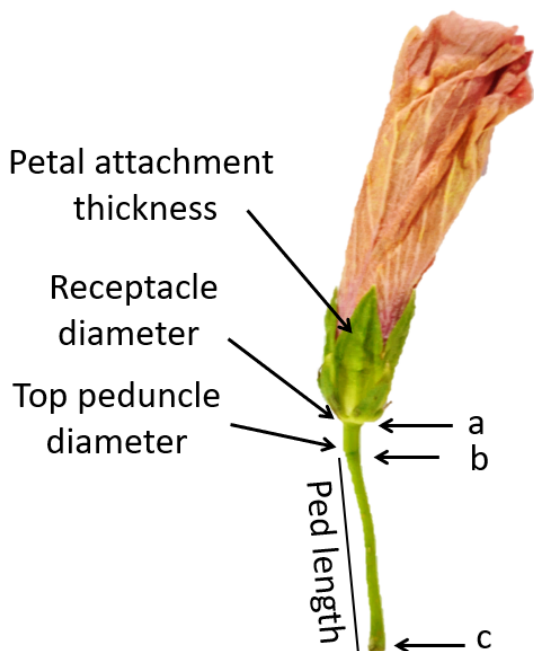


Figure 4: Floral morphological areas in *Hibiscus* assessed (ped=peduncle); black arrows denote abscission zones in *Hibiscus* flowers with a) beneath the receptacle is the corolla abscission zone, b) calyx-peduncle abscission zone; and c) separation of the peduncle to the plant.

Based on floral retention, significant differences between breeds among four morphological traits were observed. These four morphological characters are: peduncle diameter at the attachment to receptacle, peduncle length, receptacle diameter and petal thickness at the attachment site. Table 1 summarizes the means and standard deviations for the different floral morphological characters. With regards to peduncle diameter at the proximal region of the peduncle to the receptacle (Figure 5), each breed is significantly different from each other. For instance, GC had 3.21 mm, LLxEFA had 3.07 mm, RON had 1.90 mm, and WX had 1.27 mm. However, a contrasting trend was observed for the peduncle length indicating that GC had 28.36 mm, LLxEFA had 45.16 mm, RON had 61.45 mm and WX had 38.95 mm. This may suggest that peduncle length unlike the other trait is not a good determinant of floral retention length.

Characters more directly related with floral retention and its relation to floral morphology were identified. Significant differences between breeds were observed. For instance, in terms of receptacle diameter GC has the highest mean diameter with 8.49 ± 0.57 mm across the receptacle area, where the petals are attached to, followed by LLxEFA with 6.99 ± 0.52 mm. RON has 3.45 ± 0.34 mm receptacle diameter while, WX had 3.12 ± 0.32 mm (Table 1).

Significant differences were detected among the four breeds in terms of the petal thickness at the attachment site (Figure 5). At the attachment site of the petals, GC had 1.56 ± 0.57 mm petal thickness and LLxEFA had 1.12 ± 0.14 mm. RON had 0.84 ± 0.09 mm petal thickness at the attachment site while WX had 0.57 ± 0.12 mm.

Three morphological traits including: peduncle diameter nearest to receptacle, receptacle diameter and petal thickness at attachment site showed an increasing trend that is nearly uniform, except for peduncle length *ie.* GC, LLxEFA, RON and WX wherein GC has the greatest values for the mean measurements for most morphological traits, while WX has the least. However, for peduncle length, RON had the longest peduncle followed by LLxEFA, WX, and GC had the shortest peduncle. This variation in morphological characters suggests that the duration of floral retention may also vary within the typical one-day or two-day breeds.

A few morphological characters correspond with the length of floral retention including: peduncle diameter at the attachment site of the receptacle, receptacle diameter and petal thickness at the attachment site. These morphological characters tend to increase as the duration of floral retention increases from one-day to two-day old breeds (Table 1). *Hibiscus rosa-sinensis* 'Gelia Castillo' had both the highest measurements for the above-mentioned characters and had the longest floral retention. GC was followed by LLxEFA and RON, and last was WX with the lowest measurements for the characters and had the shortest duration of floral retention.

Since this trend was observed both for morphological measurements and duration of floral retention as measured through abscission of petals, this suggests that there could be a relationship between floral longevity and morphological characters.

Pearson's correlation analysis showed a significant relationship between floral retention and the three morphological characters. Characters namely: peduncle diameter, receptacle diameter and petal thickness were correlated very strongly and positively with differential floral retention across hibiscus breeds (Table 2). However, peduncle length had moderate negative correlation with duration of floral retention. The results showed that the characters having a very strong positive correlation could be used to predict breeds with longer floral retention once the measurements for these traits are available. Specifically, this suggests that hibiscus breeds with big peduncle diameter, receptacle diameter and thick petal at the attachment site will have longer flower retention on the plant. In addition, it could be emphasized further that hibiscus breeds with larger receptacle diameter will have longer flower retention as shown in hybrids GC and LLxEFA (Figure 6). In contrast, hibiscus breeds with smaller receptacle diameter like in the case of RON and WX will have short flower retention (Figure 6). During evaluation of many hybrids in a hibiscus breeding programme, the breeder can use these characteristics for quick identification of experimental hybrids with longer flower retention. This will save the breeder time and effort while evaluating large number of hybrid progenies during the peak of evaluation periods when so many flowers need to assess for flower retention. These significant associations have been first reported in this study.

Correlation and regression analyses of the length of petal retention with morphological measurements

Since Pearson's correlation analyses indicated positive relationship of few morphological characters with the duration of floral retention, prediction of petal retention of hibiscus breeds was done using regression analysis. Three linear equations depicting the assumed relationships between the two major factors such as duration of flower retention and morphological characters was depicted by a general form of $\hat{Y} = b_0 + b_1X_1$, where: $\hat{Y}$ is the estimated value for a morphological character (*e.g.*, peduncle diameter and receptacle diameter) and the amount a morphological character changes as the value for duration of retention changes and X_1 is the estimated value for

Table 1: Floral morphological characters across breeds WX, RON, LLxEFA and GC in mm. Values presented are means of thirty samples with SD.

Morphological character	Breeds			
	WX	RON	LLxEFA	GC
Peduncle Diameter nearest to receptacle	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 at the attachment site	0.57 ± 0.12	0.84 ± 0.09	1.12 ± 0.14	1.56 ± 0.20

Table 2: Pearson's correlation r-values across morphological characters versus floral retention on the plant.

Morphological character	r-value	Interpretation
Peduncle diameter	0.9236	very strong, positive
Peduncle length	-0.4545	moderate, negative
Receptacle diameter	0.9612	very strong, positive
Petal thickness at the attachment site	0.8426	very strong, positive

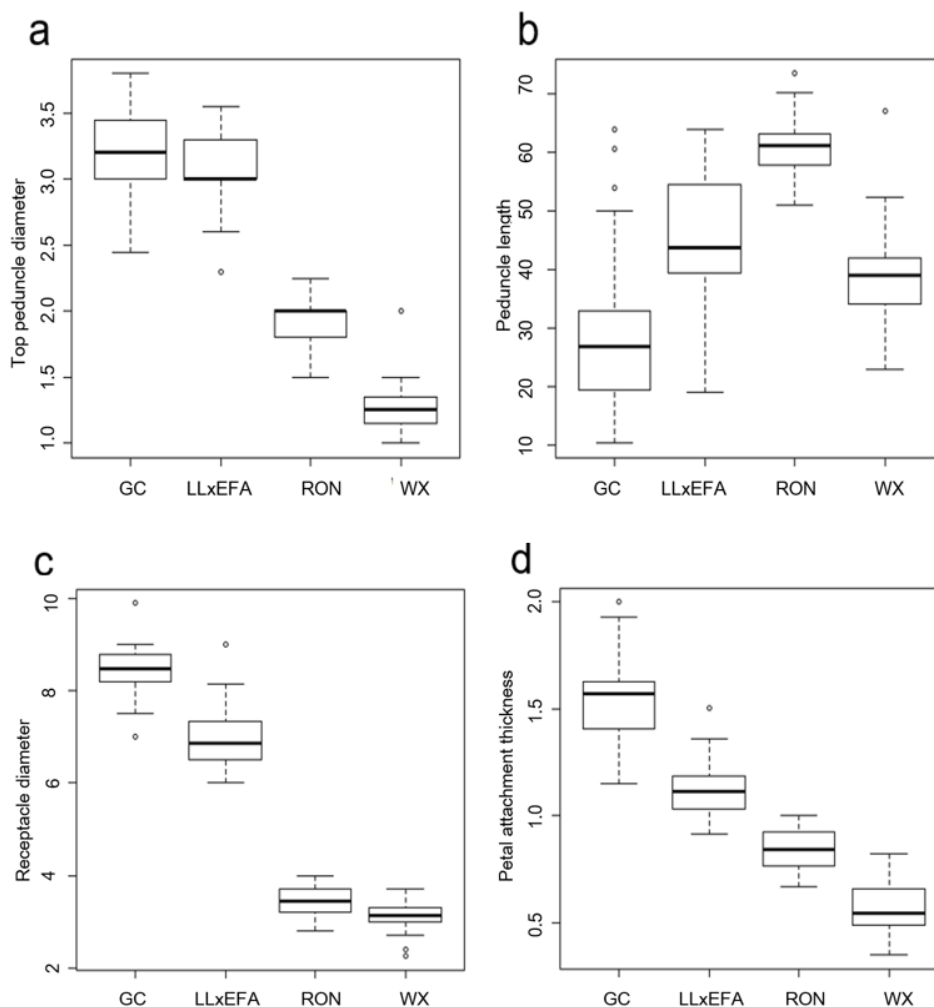


Figure 5: Box plots of the characters studied with a) top peduncle diameter b) peduncle length c) receptacle diameter and, d) petal attachment thickness.

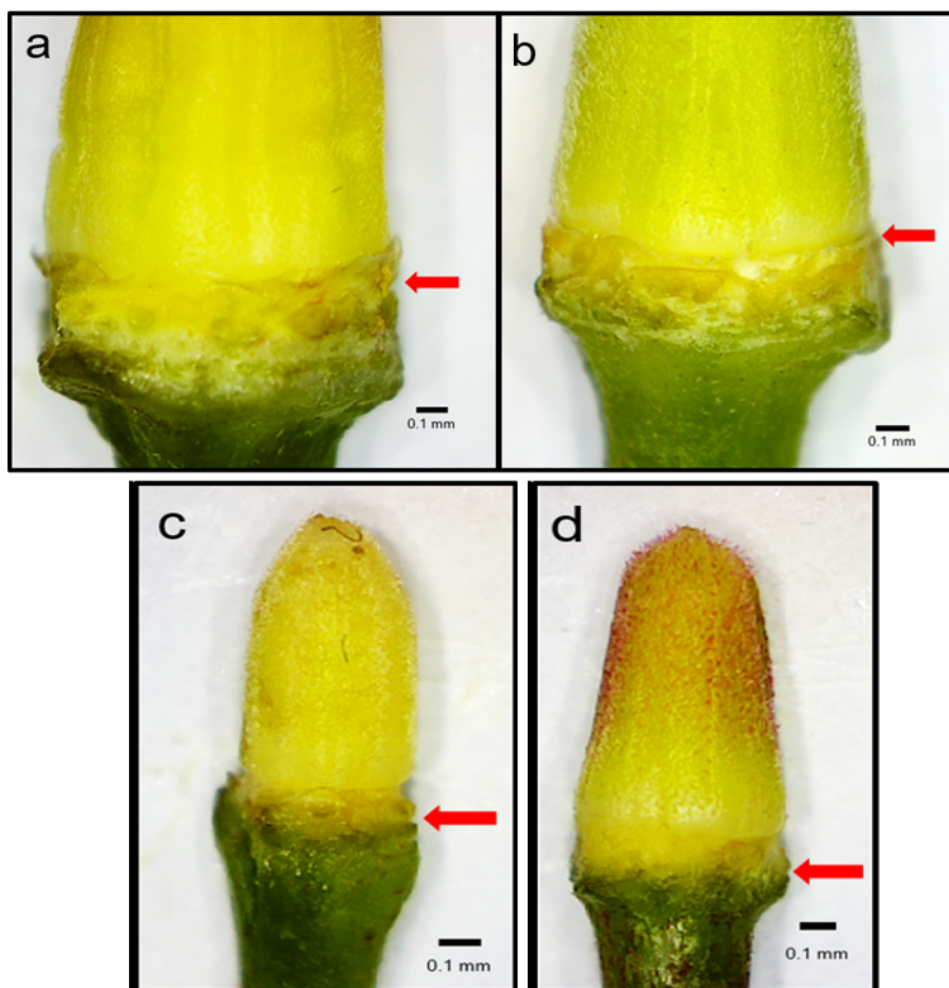


Figure 6: Exposed receptacles showing difference in receptacle diameter with thick receptacles in a) GC; and b) LLxEFA; and thin receptacle in c) RON and d) WX as receptacle diameter which was found to highly correlate with flower retention. Bar(s) approximately measure 1 mm.

the duration of floral retention on the plant. This will allow the breeders to predict the hibiscus breeds with longer duration of floral retention on the plant by knowing the morphological traits being the predictor variable.

Using the regression analyses, a coefficient of determination of 85% was obtained for peduncle diameter at the attachment of floral parts. It means that 85% of the variation in duration of floral retention is being explained by the variation in the peduncle diameter of the uppermost portion of the flower. This also indicates a high degree of linear relationship between the predictor variable (*i.e.*, morphological traits) and the criterion variable (*i.e.*, floral retention on the plant) (Figure 7). The peduncle diameter at the attachment of the floral parts in relation to the duration of floral retention is estimated by the equation $\hat{Y}=0.985+0.044X_1$ (Figure 7). This indicates that the value for the peduncle diameter at the point nearest to the receptacle, $\hat{Y}$ with a given duration of floral retention, X_1 changes by 0.044 units per unit change in X_1 , and given that X_1 is zero, the value for $\hat{Y}$ is 0.985 mm.

In addition, petal thickness at the attachment site is a morphological trait that has a high coefficient of determination of 71%, indicating that 71% of the variation in the duration of petal retention is being explained by the variation in the petal thickness of the petal at the attachment site. A high degree of relationship between the predictor variable (*i.e.*, receptacle diameter) and the criterion variable (*i.e.*, floral retention) existed. The petal thickness at the attachment site in relation to duration

of floral retention is estimated by the equation $\hat{Y}=0.442+0.018X_1$ (Figure 7). This means that the value for the petal thickness at the attachment site, $\hat{Y}$ with a given duration of floral retention, X_1 changes by 0.018 units per unit change in X_1 , given that X_1 is zero, the value for $\hat{Y}$ is 0.442 mm.

Predicting morphological measurements which may exhibit specific duration of floral retention

The very strong positive correlations between morphological characters like peduncle diameter, receptacle diameter and petal thickness with the length of floral retention suggest that floral retention is dependent on each of the three morphological characters. All morphological characters generated significant results in linear regression analyses. This allows the breeders to predict the values for morphological characters to produce a breed with longer floral retention in a cross to attain higher floral retention in *Hibiscus rosa-sinensis*. For instance, for the peduncle diameter at point nearest to the receptacle, the formulated linear model was $\hat{Y} = 0.9850 + 0.0441X_1$, where: $\hat{Y}$ is estimated value for peduncle diameter at point nearest to receptacle and X_1 = estimated duration of floral retention on the plant.

In order to estimate the values for peduncle diameter that may predict duration of floral retention to be longer, we need to substitute the values to the above equation. For example, if breeders would like to achieve a floral retention length (anthesis to abscission) of three days in a hybrid or equal to 72 hours, then the equation will be as follows $\hat{Y} = 0.9850 + 0.0441(72)$,

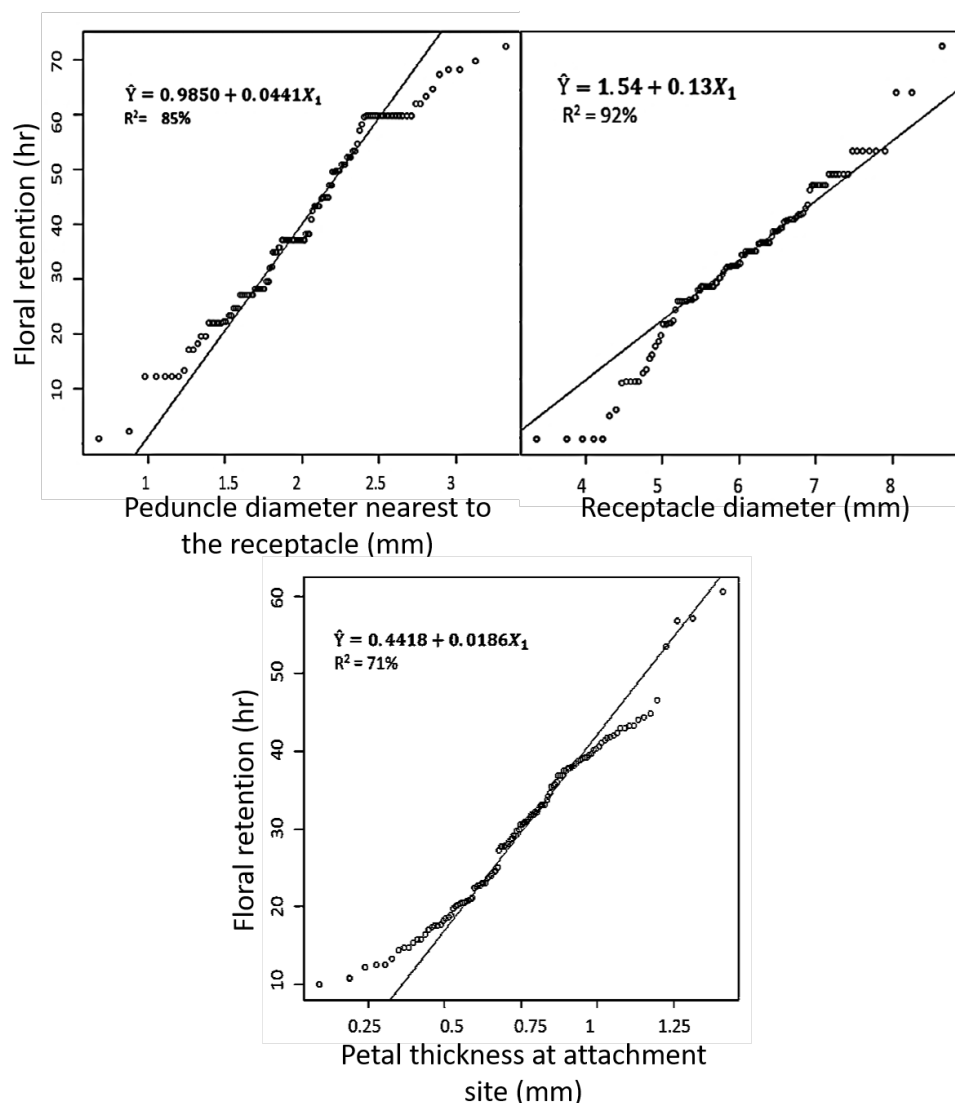


Figure 7: Estimated linear regression between floral retention and morphological traits: peduncle diameter nearest to the receptacle (mm), receptacle diameter (mm) and petal thickness at attachment site (mm).

indicating that we substituted the target number of hours for floral retention to the equation, for example, three days (72 hr), therefore $\hat{Y} = 4.1602$.

Assuming that the equation is true, a breeder needs to select hybrids with approximately 4.1602 mm of peduncle diameter at the uppermost portion to develop breeds exhibiting 72 hr flower retention on the plant. If the same procedures will be employed to compute for receptacle diameter of the three-day old breeds, breeders need to find a breed with 10.90 mm diameter across the receptacle area through the model $\hat{Y} = 1.54 + 0.13X_1$. For the petal thickness at the attachment site of the petal to the receptacle, using the linear model $\hat{Y} = 0.4418 + 0.0186X_1$. A petal thickness of 1.78 mm at the attachment site is necessary for a breed in order to exhibit 72 hr petal retention on the plant.

In the phenotypic evaluation of many hybrid progenies of hibiscus from cross-pollination, the process of evaluation will be facilitated with the use of these models, once validated. These models could help the breeders in identifying the progenies with longer floral retention and evaluation will be focused only on few characters definitive of floral retention. In addition, the laborious and time-consuming task of observing the duration of floral retention will be offset with the use of these predictive models.

Among the characters evaluated, it is most recommended to use the receptacle diameter as a reliable indicator of longer petal retention on the plant due to the highest coefficient of determination of 92% among the three phenotypic traits as revealed by regression analysis. The receptacle's high correlativity to flower retention holds reasonable because petals are attached to main floral organs through the receptacle. In consideration of its abscission mechanics, the receptacle serves as a support structure to the petals and it is where abscission proceeds.

SUMMARY AND CONCLUSION

The study aimed to investigate the phenotypic traits of one-day and two-day old hibiscus and their relationship to floral retention on the plant through correlation but not necessarily, causation. This relationship if established can be used for predicting hibiscus hybrids that will exhibit longer flower retention. Four hibiscus breeds including: 'Gelia Castillo' (GC), 'Loren Legarda' x 'Estrella F. Alabastro' (LLxEFA), 'Reddy-or-Not' (RON), and 'Wilcox' (WX) were used.

Phenotypic traits like peduncle diameter, receptacle diameter and petal thickness differentiate the four breeds from each other

with varying duration of petal retention on the plant. The general trend for the values of these phenotypic traits from the four breeds with the longest duration of floral retention to the one with the shortest floral retention is as follows: *GC* > *LLxEFA* > *RON* > *WX*.

Similarly, this trend was also observed with these four breeds for their duration of floral retention. *GC* had the longest retention with 51 hr, while *LLxEFA* with 47 hr. The two remaining breeds had short duration of floral retention with *RON* having 14 hr while *WX* had 13 hr.

Floral retention length and the morphological characters of the four breeds were correlated. The morphological characters having a 'very strong' positive correlation with floral retention length included: receptacle diameter, peduncle diameter and petal thickness. However, peduncle length is negatively correlated with floral retention length. The characters that had strong linear association with floral retention length were subjected to Linear Regression Analysis to establish prediction equations. Among the four characters, the model generated for receptacle diameter, *i.e.*, $\hat{Y}=1.54+0.13X_1$ had the highest coefficient of determination of 92%. It is most recommended to use the receptacle diameter as a definitive trait in identifying the length of floral retention in *Hibiscus*. The predicting equation above involving this trait could be used in identifying, selecting and predicting hybrid progenies of hibiscus crosses with longer floral retention length.

Three morphological characters including: peduncle diameter at point nearest to the receptacle, receptacle diameter and petal thickness at attachment site had strong positive correlation with the duration of petal retention. The characters having strong linear relationship with floral retention when subjected to linear regression analysis gave a very high coefficient of determination of 92% particularly receptacle diameter that generated the model trends. Among all of the characters, the model generated for receptacle diameter, *i.e.*, $\hat{Y}=1.54+0.13X_1$. It is then recommended to use the receptacle diameter as a definitive trait in identifying duration of floral retention in *Hibiscus* hybrids. Further studies on other breeds are necessary in assessing the scope and predictive ability of the mathematical models provided.

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