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Quantile Regression Facilitates Simultaneous Selection of Negatively Correlated Floral Traits among BC_1F_1 Progeny of Male-fertile Hybrid *Hibiscus* Cultivars Lohengrin and Resi (*H. syriacus* × *H. paramutabilis*)

Hsuan Chen[1]
Department of Horticulture, 4017 Agricultural and Life Sciences Building, Oregon State University, Corvallis, OR 97331-7304

Lan Xue[2]
Statistics Department, Oregon State University, Corvallis, OR 97331-7304

Tong Li[3]
Mechanical, Industrial, and Manufacturing Engineering, Oregon State University, Corvallis, OR 97331-7304

Ryan N. Contreras[2,4]
Department of Horticulture, 4017 Agricultural and Life Sciences Building, Oregon State University, Corvallis, OR 97331-7304

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Abstract. ***Hibiscus syriacus*** **is a woody shrub in the Malvaceae family that is common in landscapes due to its broad adaptability and variable ornamental characteristics. Interspecific hybridization has been used to improve** ***Hibiscus*** **by building novel floral traits, hybrid vigor, and hybrid infertility. A few interspecific hybrid** ***Hibiscus*** **cultivars (*H. syriacus* × *H. paramutabilis*), such as Lohengrin and Resi, are notable because of their vigorous vegetative growth, female infertility, and large flowers. However, little is known about the male fertility and breeding potential of these hybrid cultivars, which could increase flower size by backcrossing to** ***H. syriacus*****. In this study, we estimated male fertility of the two hybrid cultivars by acetocarmine staining and in vivo pollination and assessed selection methods for floral traits, specifically flower size and petal number. A BC_1F_1 population of 294 individuals was developed by crossing hybrid cultivars Lohengrin or Resi with a variety of double-flowered** ***H. syriacus*** **cultivars. A negative correlation between petal number and petal area was detected by quantile regression, which is a method that circumvents the problem of simple linear regression, which violates statistical assumptions. Quantile regression was used to build simultaneous selection thresholds for different levels of required stringency. As expected, the female fertility of hybrid cultivars was extremely low or zero; however, the male fertility of hybrid cultivars was not reduced compared with** ***H. syriacus*** **cultivars. A negative linear correlation between the petal number and petal area of the BC_1F_1 individuals was observed. In addition, quantile regression was recommended to set a single selection threshold to be applied to the selection of two negatively correlated traits, which was more effective than independent selection of petal numbers and petal areas among progeny.**

Hibiscus, a genus in the Malvaceae family, contains ≈300 species of trees, shrubs, and herbs with various growth habits. Due to their impressive flowers and long flowering season, many *Hibiscus* species have been used as ornamental plants. Most *Hibiscus* species are tropical, but *Hibiscus syriacus* is widely used in temperate regions due to its broad adaptability and variable ornamental characteristics. *H. syriacus* is the most commonly used hardy *Hibiscus* species in temperate to subtropical zones due to its cold tolerance and persistent, woody stems (Lawton, 2004). Consumer demand for novelty and color has driven most of the breeding efforts toward improving the floral traits of *H. syriacus* and other ornamentals.

Interspecific hybridization is an important breeding strategy that has been used for many ornamental crops (van Tuyl and Lim, 2003); in addition, it has been a common strategy for cultivar development of *Hibiscus* with novel phenotypes (Ha et al., 2014, 2015; Klips, 1999; Kuligowska et al., 2016; Pounders and Sakhanokho, 2016; van Huylenbroeck et al., 2000; van Laere et al., 2007). Several North American herbaceous perennial species of *Hibiscus*, including *H. coccineus*, *H. laevis*, and *H. moscheutos*, are reportedly compatible (Klips, 1999; Kuligowska et al., 2016). A multiple-species hybrid cultivar Blue Angel (*H. ×moscheutos*) that showed wider adaptability and a longer flowering season (from early summer to late fall) compared with its parents (Malinowski et al., 2012) was released. In addition to interspecific crosses within a section,

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[1] Graduate Research Assistant.

[2] Associate Professor.

[3] Graduate student.

[4] Corresponding author. Email: ryan.contreras@oregonstate.edu.

H. mutabilis has been used for intersectional crosses with *H. moscheutos*. Three interspecific hybrid cultivars of *H. mutabilis* × *H. moscheutos* that were recently released showed an extended flowering season, better cold adaptability, increased vigor, and infertility (Pounders and Sakhanokho, 2016).

Interspecific hybridization has been applied to improve *H. syriacus*. Two related species, *H. sinosyriacus* and *H. paramutabilis*, are compatible with *H. syriacus* (van Huylenbroeck et al., 2000; van Laere et al., 2007). The two species were used to increase flower size, alter leaf morphology, and increase vigor (van Laere et al., 2007). Early research reported that interspecific hybridization between *H. syriacus* and the other two species could be accomplished only if *H. syriacus* was used as the female parent (van Laere et al., 2007); however, recent reports showed that this barrier is not impregnable. Daewangchun is a cultivar with a unique eyespot that was created and released using *H. syriacus* 'Samchully' as the female parent and *H. sinosyriacus* 'Seobong' as the male parent (Ha et al., 2015). Another new interspecific hybrid cultivar, Tohagol Red, resulted from the reciprocal cross combination (Ha et al., 2014). Both hybrid cultivars showed some advantages attributable to interspecific hybridization; however, crosses between *H. sinosyriacus* and *H. syriacus* do not appear to be a good strategy for creating infertile cultivars because Daewangchun and Tohagol Red are both fertile (Ha et al., 2014, 2015). However, interspecific hybridization between *H. syriacus* ($2n = 4x = 80$) and *H. paramutabilis* ($2n = 4x = 82$) was viable only when *H. syriacus* was used as the female parent (van Laere et al., 2007; van Tuyl and Lim, 2003). Furthermore, F_1 hybrids were observed to have low self-fertility, and only a few F_2 progeny were obtained (van Laere et al., 2007). Based on acetocarmine staining, F_1 hybrids had 65% to 88% stainable pollen, which was slightly lower than that of the parents, which had 90% to 99% stainable pollen (van Laere et al., 2009); however, the in vivo pollination success rate and male fertility level of the F_1 hybrids (*H. syriacus* × *H. paramutabilis*) remain unknown.

The fertility information of cultivars and breeding materials is important. For breeding projects, fertility information helps breeders to design the breeding programs and hybridization strategies. When breeding ornamental plants, breeders invest much effort in creating low-fertility cultivars to reduce the possibility of escaping cultivation or becoming invasive (Gagliardi and Brand, 2007; Oates et al., 2014). In addition, infertile plants might spend more energy on vegetative growth and bloom instead of producing seeds, which are a priority after flowering. Based on their morphological characteristics, *Hibiscus* cultivars like Lohengrin, Resi, and Tosca are believed to be hybrids of *H. syriacus* × *H. paramutabilis* (Dirr, 2009). These three hybrid cultivars are all notable for their large flowers, large leaves, and low fertility, with almost no fruit set. However, the male fertility of these hybrid cultivars is unknown. Pollen staining using acetocarmine has been regularly used in studies of woody plants, even though it is understood that it provides an overestimation. For example, Contreras et al. (2007) observed that allotetraploid *Rhododendron* had 68% pollen staining but 45% in vitro pollen germination. Regardless of the potential for overestimation, acetocarmine staining is a useful tool for rapidly and simply testing male fertility, and it is most useful when compared with a fully fertile control.

Flower size, petal number, flower color, and flower shape are important ornamental plant attributes because these traits directly influence the consumer appeal. Independent selection of two correlated floral traits may lead to biased and unpredictable or low selection rates. For example, selection based on two independent thresholds of negatively correlated traits might result in a very low selection rate because plants with one excellent trait tend to have poor other traits; therefore, very few plants would satisfy both thresholds and be selected. In this case, a simultaneous selection threshold using a regression line of correlated traits may overcome this problem. However, using simple regression to build a selection threshold can be problematic. The simple linear model is only useful for building a threshold with a selection rate of 0.5 because simple linear regression is based on mean values of the response variable such that 50% of data points are expected to be above the regression line. However, for different breeding purposes, various selection rates might be required; generally, they will be much lower than 50%. Manually shifting the regression line until the required proportion of data points pass the threshold may fail to correct this problem, particularly when the simple linear regression model assumptions are violated. For example, when the residual variation is not constant, the shifted simple linear regression line might not be useful because the threshold is influenced by data points with larger variances, which is very common in breeding projects.

Quantile regression is a useful method of setting thresholds for various selection rates because it is more resistant to model assumption violations. Quantile regression was first introduced by Koenker and Bassett (1978), and it has been recently used in ecological and agricultural research to estimate relationships or effects between two variables (Cade and Noon, 2003; Casagrande et al., 2010). Unlike simple linear regression that only estimates the relationship between an explanatory variable and the conditional mean values of the response variable, quantile regression can estimate all possible relationships between an explanatory variable and various quantiles of the response variable (Cade and Noon, 2003). Furthermore, quantile regression has been recommended for analyzing biological data with unequal variance (Cade and Noon, 2003). As such, quantile regression could be a useful tool in datasets with a complex distribution and/or when the assumptions of normality required for simple linear regression are violated.

The objectives of the current report were to assess the male fertility of interspecific hybrid *Hibiscus* cultivars Lohengrin and Resi and to develop appropriate selection methods to optimize simultaneous selection for larger flowers and increased petal numbers.

Materials and Methods

Plant material. Ten *Hibiscus* cultivars, including eight double-flower *H. syriacus* cultivars and two interspecific hybrid cultivars of *H. syriacus* × *H. paramutabilis* (Table 1), were used for in vitro pollination tests and to create a hybrid BC_1F_1 population during Summer 2015. A total of 294 hybrid plants from all viable cross combinations were grown in 15-L containers filled with 100% unaged douglas fir (*Pseudotsuga menziesii*) bark (Lane Forest Products, Eugene, OR). Media was amended with 20N–2.6P–10.0K controlled-release fertilizer with micronutrients (Haifa Chemicals, Savannah, GA) incorporated at 7 kg·m^{-3}, a granular starter charge of 5N–0.4P–3.3K with lime (Nursery Connection, Hubbard, OR) incorporated at 10 kg·m^{-3}, and granular bifenthrin (FMC Corp.,

Table 1. Cross combinations and progeny resulting from crosses among *Hibiscus syriacus* cultivars and interspecific hybrids Lohengrin and Resi.

Female	Male	Pollinations (no.)	Capsules (no.)	Fruits per pollination (%)[z]	Seeds (no.)	Seedlings (no.)	Seedlings (no./pollination)	Seedlings used (no.)
H. syriacus 'Lavender Chiffon'	*H. syriacus* 'Strawberry Smoothie'	4	3	75.00	74	71	17.75	-
H. syriacus 'Lavender Chiffon'	*H. syriacus* 'White Chiffon'	3	2	66.67	40	40	13.33	-
H. syriacus 'Lavender Chiffon'	*H. syriacus* 'Raspberry Smoothie'	5	0	0.00	-	-	-	-
H. syriacus 'Raspberry Smoothie'	*H. syriacus* 'Lavender Chiffon'	4	2	50.00	8	0	-	-
H. syriacus 'Raspberry Smoothie'	*H. syriacus* 'Strawberry Smoothie'	4	1	25.00	4	0	-	-
H. syriacus 'Raspberry Smoothie'	*H. syriacus* 'White Chiffon'	9	3	33.33	8	0	-	-
H. syriacus 'Strawberry Smoothie'	*H. syriacus* 'Lavender Chiffon'	2	2	100.00	36	13	6.50	-
H. syriacus 'Strawberry Smoothie'	*H. syriacus* 'Raspberry Smoothie'	2	0	0.00	-	-	-	-
H. syriacus 'Strawberry Smoothie'	*H. syriacus* 'Sugar-tip'	1	0	0.00	-	-	-	-
H. syriacus 'Strawberry Smoothie'	H2014-253-07	1	1	100.00	5	0	-	-
H. syriacus 'Sugar-tip'	*H. syriacus* 'White Chiffon'	1	1	100.00	2	0	-	-
H. syriacus 'White Chiffon'	*H. syriacus* 'Raspberry Smoothie'	8	1	12.50	5	0	-	-
H. syriacus 'White Chiffon'	*H. syriacus* 'Strawberry Smoothie'	2	2	100.00	65	62	31.00	-
H. syriacus 'White Chiffon'	*H. syriacus* 'Sugar-tip'	3	3	100.00	12	8	2.67	-
H. syriacus[y]	***H. syriacus***	**49**	**21**	**42.86 ± 13.86**	**259**	**194**	**3.46**	**-**
H. syriacus 'Lavender Chiffon'	'Lohengrin'	43	11	25.58	**115**	59	1.37	24
H. syriacus 'Blue Chiffon'	'Lohengrin'	13	3	23.08	**12**	6	0.46	0
H. syriacus 'Blushing Bride'	'Lohengrin'	21	11	52.38	**307**	247	11.76	65
H. syriacus 'Pink Chiffon'	'Lohengrin'	12	10	83.33	73	27	2.25	15
H. syriacus 'Raspberry Smoothie'	'Lohengrin'	42	7	16.67	17	15	0.36	7
H. syriacus 'Strawberry Smoothie'	'Lohengrin'	85	31	36.47	417	186	2.19	66
H. syriacus 'White Chiffon'	'Lohengrin'	10	10	100.00	237	175	17.50	56
H. syriacus	**'Lohengrin'**	**226**	**83**	**36.72 ± 6.45**	**1178**	**715**	**2.97**	**233**
H. syriacus 'Blue Chiffon'	'Resi'	3	3	100.00	51	29	9.67	19
H. syriacus 'Blushing Bride'	'Resi'	5	2	40.00	30	21	4.20	18
H. syriacus 'Lavender Chiffon'	'Resi'	11	5	45.45	17	15	1.36	11
H. syriacus 'Pink Chiffon'	'Resi'	5	1	20.00	3	3	0.60	0
H. syriacus 'Raspberry Smoothie'	'Resi'	10	1	10.00	1	1	0.10	1
H. syriacus 'Strawberry Smoothie'	'Resi'	10	0	0.00	-	-	0.00	0
H. syriacus 'White Chiffon'	'Resi'	5	5	100.00	18	14	2.80	10
H. syriacus	**'Resi'**	**49**	**17**	**34.69 ± 13.33**	**120**	**83**	**1.56**	**59**
'Lohengrin'	*H. syriacus* 'Blue Chiffon'	8	0	0.00	-	-	-	-
'Lohengrin'	*H. syriacus* 'Blushing Bride'	12	1	8.33	2	2	0.17	2
'Lohengrin'	*H. syriacus* 'Lavender Chiffon'	27	0	0.00	-	-	-	-
'Lohengrin'	*H. syriacus* 'Strawberry Smoothie'	5	0	0.00	-	-	-	-
'Lohengrin'	*H. syriacus* 'White Chiffon'	8	0	0.00	-	-	-	-
'Lohengrin'	***H. syriacus***	**60**	**1**	**1.70 ± 3.69**	**2**	**2**	**0.03**	**2**
'Resi'	*H. syriacus* 'Blushing Bride'	2	**0**	0.00	-	-	-	-
'Resi'	*H. syriacus* 'Raspberry Smoothie'	1	**0**	0.00	-	-	-	-
'Resi'	*H. syriacus* 'Strawberry Smoothie'	2	**0**	0.00	-	-	-	-
'Resi'	*H. syriacus* 'White Chiffon'	1	**0**	0.00	-	-	-	-
'Resi'	***H. syriacus***	**6**	**0**	**0.00**	**-**	**-**	**-**	**-**
'Lohengrin'	**'Lohengrin'**	**14**	**0**	**0.00**	**-**	**-**	**-**	**-**
Total								**294**

[z] 95% confidence interval.

[y] Bolded rows represent the total (pollinations, capsules, seeds, seedlings, and seedlings used) or mean (fruits per pollination, seedlings per pollination) for that cross combination type. For example, *H. syriacus* × 'Lohengrin' represents the compilation of the data for the seven female cultivars of *H. syriacus* crossed with 'Lohengrin' as the male parent.

Philadelphia, PA) incorporated at 0.24 $kg{\cdot}m^{-3}$. Plants were grown at the Lewis-Brown Farm in Corvallis, OR (USDA zone 8b, lat. 44.552979°N, long. 123.218964°W) and phenotyped during Summer 2017.

In vivo fertility. Interspecific hybrid cultivars Lohengrin and Resi were used as pollen parents to backcross to the eight *H. syriacus* cultivars for generating the BC_1F_1 population and to determine their male fertility. Reciprocal crosses were conducted between the hybrid cultivars and double-flower *H. syriacus* cultivars to assess female fertility. The results of the in vivo fertility test were summarized by *H. syriacus* × *H. syriacus*, *H. syriacus* × 'Lohengrin', *H. syriacus* × 'Resi', 'Lohengrin' × *H. syriacus*, 'Resi' × *H. syriacus*, and hybrid cultivar × hybrid cultivar (Table 1). No self-pollination was included based on prior observations of self-incompatibility of *H. syriacus* cultivars (Lattier, 2017). The number of pollinations, fruit set, seed number, seed per pollination, and seedlings per pollination were recorded (Table 1). Pollination rates and their 95% confidence intervals of the hybrid cultivars and the *H. syriacus* cultivars were estimated by the binomial two-proportion z-test.

In vitro pollen stainability test. In vitro pollen stainability of 'Lohengrin', 'Resi', and *H. syriacus* 'Red Heart' were tested by acetocarmine staining. Mature pollen was collected from flowers on the first day of the 2-d flowering period (Lattier, 2017) and spread on a clean slide. A drop of 2% acetocarmine was spread with pollen before the cover glass was applied. Pollen was observed under magnification ×100 using a compound light microscope (Axio Imager A1; Zeiss Microscopy, Oberkochen, Germany), and stainability (as a proxy for relative fertility) was assessed by counting the number of stained and unstained pollen. Stainable pollen proportions and their 95% confidence intervals were estimated by the binomial two-proportion z-test.

Phenotyping floral traits and quantile regression. Floral traits including petal number and petal area (petal length × petal width) were evaluated using two flowers of each plant at anthesis. The petal area was reported as an indirect indicator representing flower size because the angle of petal attachment often skews the direct diameter measurement of flowers (Lattier, 2017). Petal area was the response variable, Y, and petal number was the explanatory variable. The relationship between the petal number and petal area was tested by both simple regression and quantile regression. Statistical assumptions were violated for the simple linear regression method. Quantile regression uses the value of the τ-th ($0 < \tau < 1$) Y to make a regression line for the random variable Y, and the proportion of Y values that are smaller than or equal to the value of the random variable Y is τ (Casagrande et al., 2010). When within a certain range of τ, if the confidence intervals of the slope were observed to not cover zero, then a linear relationship between the two variables in the range would exist as defined. When making a simultaneous selection, regression lines of different τ values were used to make selection thresholds at 1-τ selection rates. For example, the possibility of data points below the regression line of $\tau = 0.9$ was 90%. If a selection was targeted at a selection rate of 0.1, then the quantile regression line of $\tau = 0.9$ would be recommended as the threshold. Therefore, the top 10% of petal area plants among all different petal numbers will be selected.

The quantile linear regression formula was defined as $Y \sim \beta_0 + \beta_1 X + \varepsilon$. R package quantreg was used to process the quantile regressions for this study. Intercepts (β_0) and slopes (β_1) of each τ quantile value ($\tau = 0.05, 0.10, 0.15, \ldots, 0.95$) and 90% confidence intervals of the intercept and slope were estimated using R. Estimated quantile regression lines for all τ values were included (Fig. 1). Visualized confidence interval distributions of slopes and intercepts for all quantiles were created by R package ggplot2 (Fig. 2). Simultaneous selection thresholds and selection results were estimated by the newly built R function quick_rqselect. R function quick_rqselect was built and designed for generating an automatic output of selection

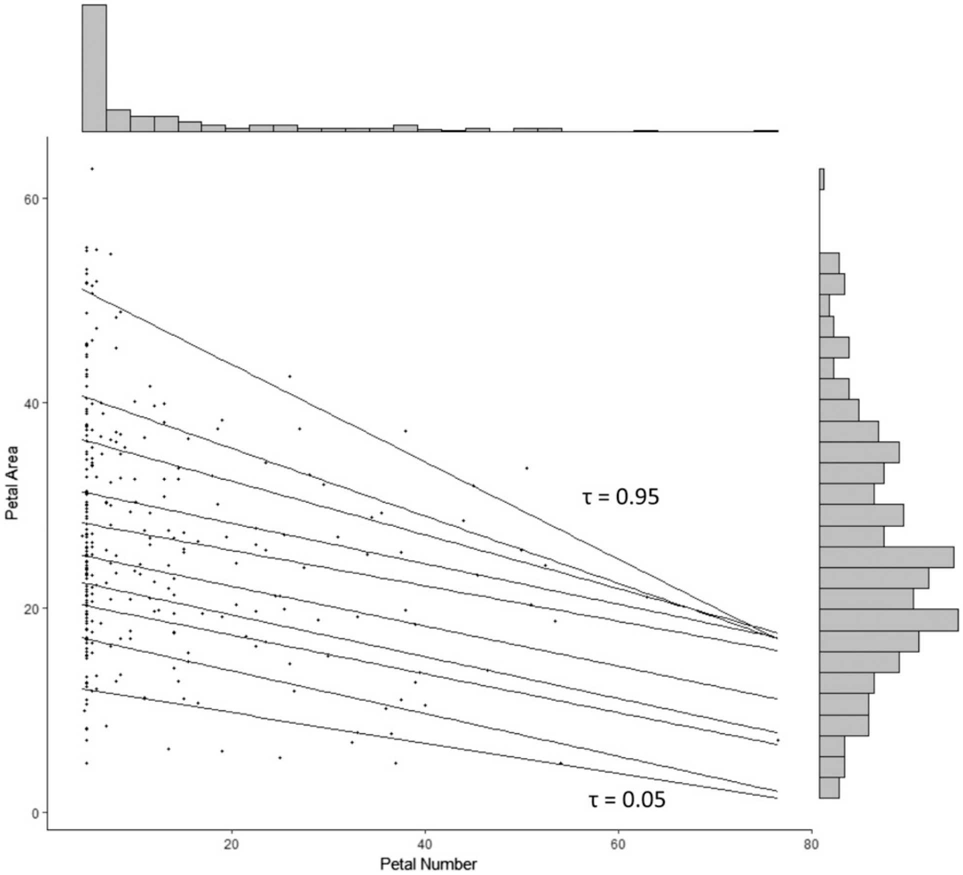

Fig. 1. Petal area and petal number plotted with fitted quantile regression lines (from $\tau = 0.05$ to $\tau = 0.95$) for the simple linear model, $Y \sim \beta_0 + \beta_1 X + \varepsilon$, and the distributions of the petal area (right) and petal number (upper) among 294 BC_1F_1 progeny from crosses between several *Hibiscus syriacus* cultivars and two interspecific hybrids Lohengrin and Resi.

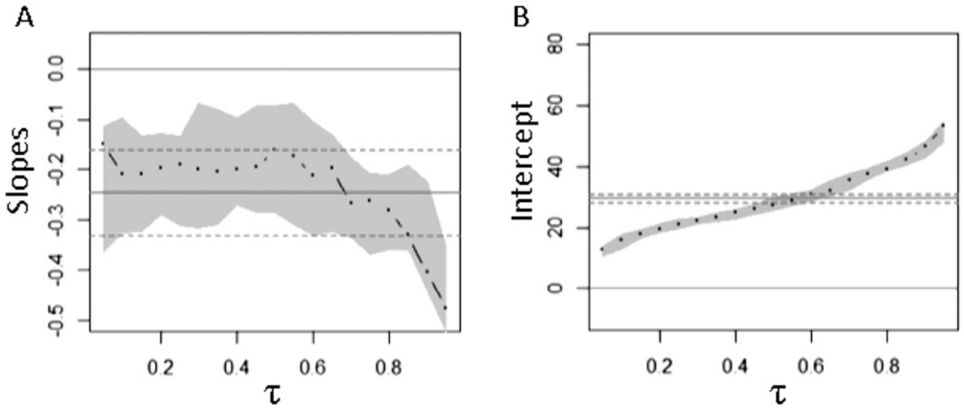

Fig. 2. Parameter estimates for the simple linear model, $Y \sim \beta_0 + \beta_1 X + \varepsilon$, for quantile regression (from $\tau = 0.05$ to $\tau = 0.95$) relating the petal number to the petal area among 294 BC_1F_1 progeny from crosses between several *Hibiscus syriacus* cultivars and two interspecific hybrids Lohengrin and Resi. The dotted line represents estimated values of the parameter, and the gray area indicates 90% confidence intervals of the respective quantile rank-score tests (from $\tau = 0.05$ to $\tau = 0.95$, with increments of 0.05). Solid line and dashed lines indicate the estimate values and 90% confidence intervals of parameters for simple linear regression.

Table 2. In vitro pollen stainability test of two interspecific *Hibiscus* hybrids ('Lohengrin' and 'Resi') and *H. syriacus* 'Red Heart' assessed using 2% acetocarmine.

Cultivar	Stained pollen (no.)	Unstained pollen (no.)	Total pollen (no.)	Stained pollen [± CI (%)]^z
Red Heart	1034	99	1133	91.26 ± 1.64
Lohengrin	1281	231	1512	84.72 ± 1.81
Resi	419	618	1037	41.41 ± 2.99

^{z}95% confidence interval.

results from the chosen τ ($\tau = 0.05, 0.15 \ldots, 0.95$) quantile regression thresholds. The built function quick_rqselect generated the simultaneous selection results that indicated whether a plant passed the threshold of the chosen τ value (selection rate = 1- τ) for our breeding process. Individual plants that passed the threshold of the chosen selection rate (1-τ)% were noted as "yes" in an extra column of an output CSV file.

Results

Fertility test. Pollen stainability rates of *H. syriacus* 'Red Heart' (91%), 'Lohengrin' (85%), and 'Resi' (41%) were significantly different ($P < 0.05$ after Bonferroni correction) (Table 2). Two hybrid cultivars, Lohengrin and Resi, were female-infertile, with only one fruit set and two seeds obtained from 60 pollinations with Lohengrin as the female parent. However, when used as male parents, there was no significant difference in the seedling number per pollinated flower among hybrid cultivars and the *H. syriacus* cultivars (Table 1), even though the percent of stainable pollen was reduced for Resi (41%) compared with Lohengrin (85%). Although the chances to recover the fruit set from each pollination of the three crossing categories were similar, the seedling number per pollination varied. The average seedling numbers per pollination of *H. syriacus* × *H. syriacus*, *H. syriacus* × 'Lohengrin', and *H. syriacus* × 'Resi' were 3.46, 2.97, and 1.56, respectively (Table 1). The reduction in the percent of stainable pollen may not impact the chance of recovering the fruit set from each pollination, but it may be responsible for the observed reduction in seedlings per pollination.

Phenotype of the BC_1F_1 population. Wide ranges of petal areas and petal numbers were observed in the BC_1F_1 interspecific hybrid populations. The petal area ranged from 4.73 to 62.90 cm^2, and the distribution had a bell shape with a longer upper tail (Fig. 1). The petal number ranged from 5 to 76 petals, with an exponential distribution (Fig. 1). Most individuals showed either a single flower (petal number = 5) or petal numbers from 6 to 10 (Fig. 1). Simple linear regression showed the negative linear correlation between the petal area and petal number, with a slope of –0.246 ($P < 0.0001$). The 95% confidence interval of the slope was estimated as –0.349 to –0.144 (Fig. 2). The confidence interval of the slope in the simple linear regression not covering zero might imply the existence of a negative correlation between the petal area and petal number. However, in our data, at least one statistical assumption, that of equal variance, was violated. Petal area variances for each petal number gradually decreased while the petal number increased. Violating the assumption of equal variances might lead to an inappropriate result because the predicted confidence interval of the slope might not be accurate.

Quantile regressions portrayed the negative correlation between the petal area and petal number and circumvented the unequal variance problem (Figs. 1 and 2). The fitted response lines corresponding to the linear model for the considered values of τ (from $\tau = 0.5$ to $\tau = 0.95$) were included (Fig. 1). The line showing the largest petal area values corresponded to the quantile $\tau = 0.95$, and the line showing the smallest petal areas corresponded to the quantile $\tau = 0.05$. Theoretically, there were ≈5% and 95% individuals with petal area values that were smaller than the value indicated by the two lines of τ values 0.05 and 0.95. The 90% confidence intervals for all quantiles of different τ values ($\tau = 0.05, 0.10, 0.15, \ldots, 0.95$) showed that the effect of the petal number was significantly different from zero in all quantiles. The 90% confidence intervals for all τ-th quantile slopes were not significantly different from those of the slope of the simple linear regression model until τ was higher than 0.95 (Fig. 2A). Quantile regression estimates indicated a negative linear relationship with all quantiles of different τ values [$P \leq 0.1$ for H0 (null hypothesis): slope = 0 (Fig. 2)] of petal areas across petal numbers.

Discussion

In vitro pollen stainability test and in vivo fertility test. All three tested cultivars produced a moderate to high percent of stainable pollen, indicating they could be potentially fertile as a male parent. The statistical difference of the stainable pollen proportions between the three tested cultivars might not necessarily imply a lower reproductive success rate when used in controlled crosses. When performing artificial pollination, a large number of pollen grains are applied to the recipient stigma, but successful pollination and fertilization might need only a few stainable pollen grains. Therefore, when each artificial pollination contains enough stainable pollen to reach a maximum pollination rate, higher or lower stainable pollen proportions might not matter. In other words, small variations in stainability above a minimum threshold will not impact the success of controlled crosses because an excess of pollen is applied to each stigma.

The cross results of the in vivo test showed that both hybrid cultivars Lohengrin and Resi are male-fertile and female-infertile. Interspecific hybrid plants showing male and female fertility differences have been observed in many hybrid plant species (Yoon et al., 2006); in our case, the partial fertility might be valuable for ornamental plant breeding projects. Genetic control (Brubaker et al., 1993), abnormal ploidy in gametes such as aneuploidy or unreduced gametes (Karlov et al., 1999; Mason et al., 2011), and abnormal chromosome recombination (van Laere et al., 2009) could be involved in hybrid infertility. In many interspecific hybridizations, male fertility tends to be reduced, and hybrid plants may retain a low level of female fertility. For example, the interspecific hybrid pepper, *Capsicum annuum* × *C. baccatum*, was male-infertile but 10% female-fertile; this fertility facilitated introgression of anthracnose resistance from *C. baccatum* to *C. annuum* (Yoon et al., 2006). Interestingly, the interspecific hybrid *Hibiscus* cultivars in the present study showed extreme female infertility while remaining male-fertile (Table 1). This trend of female infertility and male fertility of interspecific hybrids has been reported for *Gossypium* (Brubaker et al., 1993) and *Lilium* (Karlov et al., 1999). For ornamental plants, female-infertile plants have been preferred due to their reduced potential to become weedy or invasive (Gagliardi and Brand, 2007; Oates et al., 2014). In addition, infertile plants might potentially spend more energy on vegetative growth and bloom instead of producing seeds, which usually is a priority after flowering. These *Hibiscus* hybrid cultivars were shown to benefit from female infertility, but breeders could still achieve further breeding manipulations by using their fertile pollen.

Plants with reduced fertility might be used to create more plants with low fertility or infertility. The low fertility of interspecific hybrids could be seen as a trait to be recovered or to be maintained by selecting for different purposes. For sexually propagated crops, fertility is important; therefore, individuals with comparatively higher fertility are intentionally selected and used in later crossings to rescue fertility in later generations. For example, hybridization between *Phaseolus vulgaris* and *P. acutifolius* resulted in low-fertility F_1, and the higher-fertility individuals in later generations were continuously selected until the fertility was fully recovered (Mejía-Jiménez et al., 1994). An interspecific hybrid population of *Trifolium uniflorum* × *T. repens* was reported to have low fertility, but fertility varied among the hybrids and was gradually recovered by intentional selection during the following generations (Naeem et al., 2017). In contrast to sexually propagated agronomic crops, sterility or low fertility is often preferred in asexually propagated crops. This is particularly true for ornamentals, which represent a greater portion of introduced plants that have escaped cultivation and have become invasive.

Under field conditions, many plants in the BC_1F_1 population showed low female fertility. When allowed to open-pollinate in the presence of fertile pollinizers and an abundance of pollinators, 75% of the BC_1F_1 population either had no fruit set or set seedless fruit during 2017, whereas control plants were very fruitful, with capsules containing many seeds in the same field (data not shown). However, long-term fertility tests need to be performed among the current population and any future progeny because various cross combinations may result in a range of fecundity. In addition, some plants did not flower until very late Sept. 2017; therefore, the low fruit number or seed number might not imply real infertility. Instead, it likely was related to the environment or lack of pollinizers and pollinators at the time of flowering. Further controlled tests of crossing with fertile parents and observations in regions that accumulate greater heat units to promote earlier flowering are needed to determine any final conclusions.

Quantile regression analysis. Quantile regression analyses are necessary to appropriately portray the relationship between the petal area and petal number. Simple linear regression detected a negative correlation between the flower size and petal area; however, our data violated a key assumption of simple linear regression because the variance was not uniform. Quantile regression does not require this assumption; therefore, it provides a more reliable result and can confirm negative correlations. This type of distribution might imply multiple unknown effects; therefore, analyzing the relationship between the measured variation and response variation would be challenging (Cade and Noon, 2003). To properly analyze data with unequal variances and describe the relationship between variables, quantile regression has been recommended (Cade and Noon, 2003; Koenker and Hallock, 2001). In this study, many potential unmeasured effects such as unknown genes, transgressive segregation, chromosome rearrangement, temperature, and environmental effects could be involved in petal area values.

Quantile regression lines for selection thresholds. In this study, quantile regressions were also used for setting simultaneous selection thresholds of two traits that were negatively correlated. All τ-th quantile slopes were not significantly different from the slope of the simple linear regression model until τ was higher than 0.95 (Fig. 2A). The difference in slopes between regular regression and quantile regression indicated that the selection threshold from the quantile regression was important with an extreme τ value. In many breeding selection scenarios, a high selection rate is applied; therefore, setting an appropriately stringent threshold is necessary. However, as stated, the threshold necessary for the desired selection rate of one trait can bias the selection for related traits, particularly negatively correlated traits. Breeders usually select plants that have better preferences for several emphasized traits. During our *Hibiscus* breeding project, floral traits were emphasized and several independent thresholds for different floral traits were used during several selections. The negative linear relationship between the petal area and petal number was demonstrated by quantile regression; therefore, separately setting two independent thresholds for the two traits would be inappropriate. When there is a negative correlation between these two traits, plants with high petal numbers tend to have a smaller petal area size (Fig. 1); therefore, no or few plants would pass both of the independent selection thresholds and result in an extremely low selection rate. However, plants with different petal numbers have their own niches in the market, but a larger flower is usually preferred. To correct the problem of the low selection rate and satisfy the requirement for a variety of petal numbers with a large flower size, building an appropriate simultaneous selection threshold targeting plants with larger petal areas among different petal numbers is required.

A simple linear regression that estimated the average petal area values among petal numbers should not serve as a simultaneous threshold because of its limited usage and bias. The selection threshold would not able to be used in any targeted selection rate other than 0.5. Manually adjusting the intercept value of the simple linear regression line or vertically shifting the threshold line might provide thresholds for various favored selection rates. However, the unequal variance of the petal area values across petal numbers might cause a bias in selections. For example, by manually shifting the simple linear regression line such that only the top 10% of individuals are selected, the threshold results in an accurate selection rate of 0.1. However, only plants with low petal numbers would be selected because plants with smaller petal numbers tend to have greater variance in petal area values (Fig. 1). Therefore, simply shifting the linear regression line to create different simultaneous selection thresholds for different selection rates is not an appropriated method.

Quantile regression lines can be used as simultaneous selection thresholds for all favored selection rates. Lines with various τ values could easily provide thresholds for selection rates of $1-\tau$. Instead of using the uniform slope, –0.246, from the simple linear regression, quantile regression lines with various τ values with different slopes should be estimated by the values of the chosen quantiles. Because of the specialized slopes, the thresholds made by different quantiles would be more appropriate for specific selection rates, regardless of unequal variances. For example, when the selection rate of 0.1 was required, the top 10 petal area plants with different petal numbers were evenly selected by the quantile regression threshold line (τ = 0.9). Although same number of plants could be selected by both threshold methods, the quantile regression-based method easily provided multiple lines to fit different required selection rates and produced unbiased or less biased selection results that fit unequal variances.

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