Relative Fertility and Ploidy Levels of Selected Rose of Sharon Cultivars

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Significance to Industry: *Hibiscus syriacus* (rose of sharon) is a popular flowering shrub in the United States. Rose of sharon is widely adaptable and provides vivid flowers late in the season, but unfortunately it produces prolific seeds and can be weedy (2). The U.S. National Arboretum (USNA) attempted to address this issue by releasing four cultivars (‘Aphrodite’, ‘Diana’, ‘Helene’, and ‘Minerva’) that were reported to be sterile (or nearly sterile) triploids (4, 5, 6, 7). However, these cultivars have been observed to produce seed. The fecundity of these cultivars raises questions that may have serious implications for future ornamental plant breeding with regard to reversion of odd-ploidy selections and restored fertility. To answer some of the questions surrounding these cultivars, reciprocal crosses were conducted among nine cultivars readily available in the trade, including three of the USNA cultivars. We also conducted flow cytometry analysis of DAPI-stained nuclei to determine relative genome sizes and infer ploidy levels for 20 cultivars. All cultivars, including the purported triploid cultivars, were both male and female fertile with the exception of ‘Lucy’, which has petaloid stamens. We determined that all cultivars included were tetraploid with the exception of ‘Pink Giant’, which was hexaploid. Our work shows that the USNA cultivars included in our study (‘Aphrodite’, ‘Diana’, and ‘Minerva’) were fertile tetraploids. It remains unclear why they appeared to have low fertility, or near sterility, at the time of evaluation and release but have apparently regained fertility. The cultivar ‘Pink Giant’ appeared to have the lowest number of seedlings per pollinated flower and was the only cultivar in our study with a ploidy level differing from the wild-type. This suggests that ploidy manipulation remains a viable option for developing truly sterile rose of sharon cultivars.

Nature of Work: Ploidy manipulation is an important tool in developing sterile forms of nursery crops. Historically, polyploids were identified based on their morphology. Thicker and darker leaves as well as twisted or malformed flowers were often used as indicators of polyploidy. However, relying on gross morphology alone can lead to misidentification. Another method used by plant breeders to identify polyploids is the measurement of stomata. Measuring stomata is useful in identifying the ploidy level of the L-I histogenic layer, but provides no information on the germ layer (L-II) from which pollen and eggs are derived. The results of using either morphology or stomata measurements may lead to erroneously identifying plants that breed as polyploids. Today, breeders have the advantage of using flow cytometry to accurately and quickly identify ploidy levels and genome sizes.

The natural form of rose of sharon is tetraploid (2*n* = 4*x* = 80). However, publications on the cultivars 'Aprhodite', 'Diana', 'Helene', and 'Minerva' state that they are the result of chromosome doubling of the diploid cultivar 'William R. Smith', resulting in a tetraploid, and then crossing with various diploid cultivars to produce sterile triploid progeny (4, 5, 6, 7). If the original treated cultivar was actually doubled, the result would have actually been an octoploid (2*n* = 8*x* = 160) and crosses with untreated tetraploid cultivars would have resulted in hexaploid progeny (2*n* = 6*x* = 120). It remains unclear if the original polyploids were identified by any other method than gross morphology, which is an unreliable method. Therefore, the ploidy level of the original purported polyploid parent plant is in doubt. One possibility is that they were cytochimeras with their L-I histogenic layer doubled, or perhaps they contained no polyploid cells if morphology alone was used to identify them.

To evaluate male and female fertility, reciprocal crosses were made by hand among nine rose of sharon cultivars grown in a glasshouse (Table 1). There was sufficient distance between the stigmas and anthers at anthesis, eliminating the need for emasculation. Flowers that were not hand pollinated did not set seed, demonstrating that recovered seedlings are the result of cross-pollination. At maturity, seeds were collected, counted, and sown within two days. Seedling numbers were recorded after germination.

Flow cytometry was conducted according to Contreras et al. (1) with the modification that tomato (*Solanum lycopersicum* 'Stupicke' 2C = 1.96 pg) (3) was used as the internal standard because its genome size was more similar to previous reports for *Hibiscus* than pea. We used three replications for each cultivar and presented the means ± SEM. We analyzed a minimum of 2,000 particles and CV% for all samples was ≤5%.

Results and Discussion: All cultivars were male and female fertile (Table 1). Female fertility ranged from 0.08 to 10.1 seedlings per pollinated flower in 'Pink Giant' and 'Aphrodite', respectively, with a mean of 3.0 for the nine cultivars. Male fertility ranged from 0.5 to 10.1 seedlings per pollinated flower in 'Pink Giant' and 'Marina' (Blue Satin®), respectively, with a mean of 2.9 for the nine cultivars. 'Lucy' was not evaluated for male fertility because it has double flowers and does not produce pollen. The purported sterile triploid cultivars included in the study, 'Aphrodite', 'Diana', and 'Minerva' were all male and female fertile. Statistical analysis was not conducted. However, the level of fertility of these cultivars appears to be similar to the other six cultivars included. A notable exception is 'Pink Giant', which produced only 0.08 and 0.5 seedlings per pollinated flower when used as a female and male parent, respectively. This reduced level of fertility can likely be explained by the fact that 'Pink Giant' has a higher ploidy level than other cultivars, which leads to increased autosyndetic pairing of homologous chromosomes.

All cultivars except 'Pink Giant' were tetraploids with genome sizes comparable to those previously reported (Table 2) (9). The cultivar 'Oiseau Bleu' (syn. 'Blue Bird') was found

to have a genome size of 2C = 4.6 pg, confirming a previous report of its genome size as 2C = 4.68 pg (9). Other research confirmed that 'Lucy', 'Oiseau Bleu', 'Red Heart', and 'Woodbridge' were 2*n* (actually 4*x*) and that 'Diana', 'Helene', and 'Pink Giant' were 3*n* (actually 6*x*) (9). The history of ploidy levels of 'Aphrodite', 'Diana', and 'Minerva' has been unclear, but the previous report suggests that 'Diana' was a homogeneous hexaploid. One possibility is that the original plants were cytochimeras that eventually stabilized at the tetraploid or hexaploid level after repeated cycles of asexual propagation. Related to this possibility, there may be various lines of descent with various ploidy levels; depending on the source, 'Diana' and other USNA cultivars may be tetraploid or hexaploid. To investigate this, we plan to test material from original plants of 'Aphrodite', 'Diana', and 'Minerva' at the USNA in addition to testing various cultivars from nurseries, gardens, and arboreta. Additional testing of these plants may elucidate the ploidy level of their composite histogenic layers to indicate why these cultivars are now fertile tetraploids.

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Table 1. Results of crossing study conducted during 2012 to estimate the relative fertility of nine rose of sharon (*Hibiscus syriacus*) cultivars.

Cultivar	Flowers pollinated (No.)	Seedlings (No.)	Seedlings per poll. flower (No.)
As female parent			
'Aphrodite'	42	423	10.1
'Blue Bird'	36	66	1.8
'Marina'	59	318	5.4
'Diana'	97	155	1.6
'Lucy'	34	27	0.8
'Minerva'	24	44	1.8
'Pink Giant'	39	3	0.08
'Red Heart'	74	212	2.9
'Woodbridge'	70	180	2.6
Mean	52.8	158.7	3.0
As male parent			
'Aphrodite'	74	55	0.7
'Blue Bird'	54	212	3.9
'Marina'	44	443	10.1
'Diana'	55	186	3.4
'Lucy'[z]	--	--	--
'Minerva'	66	222	3.4
'Pink Giant'	50	25	0.5
'Red Heart'	66	154	2.3
'Woodbridge'	66	131	2.0
Mean	59.4	178.5	2.9

[z]'Lucy' is a double-flowered cultivar that does not produce pollen, therefore could not be assessed as a staminate parent.

Table 2. Mean relative holoploid genome size (2C) estimates ± SEM and inferred ploidy levels of nine cultivars of rose of sharon (*Hibiscus syriacus*). Estimates were performed by analyzing DAPI-stained nuclei using flow cytometry using *Solanum lycopersicum* 'Stupicke' (2C = 1.96 pg) as an internal standard.

Cultivar	Trade Name	2C Genome Size	Ploidy
'Aphrodite'		4.7 ± 0.04	4*x*
'Diana'		4.7 ± 0.06	4*x*
'Lucy'		4.6 ± 0.01	4*x*
'Minerva'		4.6 ± 0.05	4*x*
'Pink Giant'		6.8 ± 0.05	6*x*
'Red Heart'		4.7 ± 0.00	4*x*
'Woodbridge'		4.6 ± 0.06	4*x*
'Blushing Bride'		4.8 ± 0.00	4*x*
'Ardens'		4.7 ± 0.04	4*x*
'Oiseau Blue'	Blue Bird	4.6 ± 0.04	4*x*
'Marina'	Blue Satin®	4.6 ± 0.03	4*x*
'America Irene Scott'	Sugar Tip™	4.7 ± 0.04	4*x*
'Notwoodone'	Lavender Chiffon™	4.7 ± 0.08	4*x*
'Notwoodtwo'	White Chiffon™	4.7 ± 0.02	4*x*
'Notwoodthree'	Blue Chiffon™	4.6 ± 0.03	4*x*
'Antong Two'	Lil' Kim™	4.7 ± 0.04	4*x*
'Minspot'	Fiji™	4.7 ± 0.06	4*x*
'Mingrand'	Hawaii ™	4.7 ± 0.05	4*x*
'Minfren'	Bali™	4.6 ± 0.01	4*x*
'Mineru'	Tahiti™	4.6 ± 0.04	4*x*