Reproductive Pathways Among Flowering Crabapples

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Significance to Industry: Information on reproductive pathways (e.g., occurrence of aneuploidy, apomixis, pseudogamy, unreduced gametes, etc.) and differing ploidy levels of cultivars is extremely valuable for use in plant breeding programs. Fertility, crossability, heritability, and expression of traits can be influenced by these factors. This research provides information on ploidy levels and reproductive pathways for a diverse collection of flowering crabapples (*Malus* spp.Mill.) and will allow for more systematic and efficient progress in the development of new improved varieties.

Nature of Work: Flow cytometry provides a rapid method for determining plant ploidy (number of complete sets of chromosomes) levels (1) and reproductive pathways (4) by discriminating between variations of apomictic, pseudogamus, and sexual seed formation. The ploidy level and related DNA content (C value) of embryo and endosperm tissue will depend on whether the embryo sac was reduced or unreduced, whether the egg and/or central cells were fertilized, whether the male gametes were reduced or unreduced, and ploidy levels of the respective parents. Sexual fertilization between diploid parents typically results in a diploid embryo and triploid endosperm. However, variation in embryo and endosperm ploidy can result from a combination of factors. These reproductive pathways can be reconstructed by considering the genome sizes (C values) of the embryo and endosperm with knowledge of the ploidy level of the maternal parent. The objective of this study was to survey and document variations in reproductive pathways among a diverse collection of flowering crabapples that vary in ploidy level.

Materials and Methods: Seed were collected in June and July, 2004 from flowering crabapples growing in an established field plot arranged as a randomized incomplete (due to loss of some trees) block experimental design with up to three replications. Three seeds (subsamples) were randomly sampled from each tree. Samples and standard tissues were chopped with a razor blade in a Petri dish containing 0.5 mL of extraction buffer (CyStain UV Precise P Nuclei Extraction Buffer, Partec, Münster, Germany). The suspension was filtered through a 50 µM filter and nuclei were stained with 1.5 mL 4',6-diamindino-2-phenylindole (DAPI) buffer (CyStain UV Precise P Staining Buffer, Partec). The suspension was analyzed using a flow cytometer (PARTEC PA-I, Partec) to determine the mean sample nuclei fluorescence relative to that of the internal standard. Approximately 5,000 nuclei were measured per sample. DNA

content of samples was calculated as: mean fluorescence value of sample x nuclear DNA content of standard/mean fluorescence value of standard. *Pisum sativum* L. 'Ctirad', with a 2C DNA content of 9.09 pg, was used as the internal standard (2). Sample C values were determined by dividing sample DNA content by the DNA content (1C) of the maternal parent. Maternal genome size and estimated ploidy level values were reported previously (3).

Results and Discussion: Open pollinated seeds demonstrated a variety of reproductive pathways including sexual and asexual modes (Tables 1 and 2). Seeds from diploid maternal parents demonstrated sexual fertilization from reduced (1C) male and female gametes, apomixis, and fertilization of reduced embryo sacs by 2C pollen (either unreduced pollen from diploids or reduced pollen from tetraploids). Seeds from triploid maternal parents demonstrated at least eight different pathway variations. Many of the seeds from triploid females appeared to result from fertilization of a reduced, ≈1.5C, aneuploid embryo sac by either 1C or ≈1.5C (aneuploid) male games. Due to variable chromosome numbers of aneuploids, these two pathways were often difficult to differentiate. Aneuploid seeds and seedlings from these pathways may have reduced viability. Other pathways observed from triploid females included apomixis, pseudogamy with unreduced embryos and fertilization of the endosperm with gametes of varying C values, and fertilization of unreduced embryo sacs with 1C male gametes. Tetraploid maternal parents demonstrated parthenogenesis of reduced embryo sacs, fertilization of reduced embryo sacs with male gametes with various C values (including apparent aneuploids), and apomixis from unreduced embryo sacs. In a number of cases, three or more substantial peaks were observed on the cytometry histograms, from diploid and triploid maternal parents, which may represent endopolyploidization or polyembryony.

This research provides information on ploidy levels and reproductive pathways of flowering crabapples and will allow for more systematic and efficient progress in the development of improved flowering crabapples.

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Table 1. Probable reproductive pathways from open pollinated seeds of *Malus spp.*

Pathway #	Embryo C Value	Endosperm C Value	Probable Reproductive Pathway
			Diploid Maternal Parents
1	2C	3C	reduced embryo sac, fertilized by 1C ♂ gamete
2	3C	4C	reduced embryo sac, fertilized by 2C ♂ gamete
3	2C	4C	unreduced embryo sac, apomixis
			Triploid Maternal Parents
4	≈ 2.5C	≈ 4C	reduced embryo sac (aneuploid), fert. from 1C ♂ gametes
5	≈3C	≈4.5C	reduced embryo sac (aneuploid), fert. from ≈1.5C (aneuploid) ♂ gametesor unreduced embryo sac, fert. of endosperm with ≈1.5C (aneuploid) ♂ gametes
6	3C	6C	unreduced embryo sac, apomixis
7	3C	7C	unreduced embryo sac, fert. of endosperm with 1C ♂ gametes, pseudogamy
8	3C	8C	unreduced embryo sac, fert. of endosperm with 2C ♂ gametes, pseudogamy
9	3C	9C	unreduced embryo sac, fert. of endosperm with 3C ♂ gametes, pseudogamy
10	3C	10C	unreduced embryo sac, fert. of endosperm with 4C ♂ gametes, pseudogamy
11	4C	7C	unreduced embryo sac, fert. from 1C ♂ gametes
			Tetraploid Maternal Parents
12	2C	4C	reduced embryo sac, apomixis
13	3C	5C	reduced embryo sac, fertilization from 1C ♂ gametes
14	≈3.5C	≈5.5C	reduced embryo sac, fertilization from ≈1.5C (aneuploid) ♂ gametes
15	4C	6C	reduced embryo sac, fertilization from 2C ♂ gametes
16	4C	8C	unreduced embryo sac, apomixis

Table 2. Approximate genome size, estimated ploidy level, and sampling of reproductive pathways found for select *Malus* taxa.

Taxa	Approximate 2C genome size (pg)	Estimated ploidy level	Apparent reproductive pathway(s)
tschonoskii (1998-242) Z	1.52[Y]	2X	1[X]
'Lanzam' (Lancelot®)	1.61	2X	1
'Red Splendor'	1.67	2X	1
'Molten Lava'	1.67	2X	1
'Sutyzam' (Sugar Tyme™)	1.67	2X	1
'Silver Drift'	1.68	2X	1,3
'Indian Summer'	1.68	2X	1
'Snow Drift'	1.68	2X	1
'JFS-KW5' (Royal Raindrops™)	1.68	2X	1
'Robinson'	1.69	2X	1
'Sinai Fire'	1.69	2X	1
'Centzam' (Centurion®)	1.70	2X	1,2,3
'Donald Wyman'	1.70	2X	1,3
'Prairifire'	1.70	2X	1
'Schmidtcutleaf' (Golden Raindrops™)	1.70	2X	1[W]
sieboldii 'Calocarpa' (1998-238)	1.71	2X	1
'Canary'	1.71	2X	1
'Adams'	1.72	2X	1[W]
'Professor Sprenger'	1.72	2X	1
'Morning Sun'	1.72	2X	1
'Pink Satin'	1.72	2X	1,3
'Hargozam' (Harvest gold™)	1.72	2X	1,3
floribunda (1998-199)	1.72	2X	1,3
'Glen Mills'	1.73	2X	1
'White Angel'	1.73	2X	1,2,3
'Sentinel'	1.73	2X	1,3
baccata 'Jackii' (1998-218)	1.73	2X	1,3
'Luwick'	1.74	2X	1
'Callaway'	1.74	2X	1
'Mazam' (Madonna™)	1.74	2X	1
'Dolgo'	1.74	2X	1,3[W]
'Baskatong'	1.74	2X	1,3[W]

Taxa	Approximate 2C genome size (pg)	Estimated ploidy level	Apparent reproductive pathway(s)
'Liset'	1.74	2X	1
'Narragansett'	1.75	2X	1
'Louisa'	1.75	2X	1,3
'David'	1.75	2X	1
'Purple Prince'	1.75	2X	1,3
'Jewelberry'	1.76	2X	1
'Cinzam' (Cinderella®)	1.76	2X	1
'Radiant'	1.77	2X	1,3
'White Cascade'	1.78	2X	1
'Doubloons'	1.78	2X	1
'Ormiston Roy'	1.82	2X	1
'Pink Princess'	2.40	3X	(4 and/or 5),7,8,11
'Adirondack'	2.48	3X	(4 and/or 5)[W]
'Silver Moon'	2.51	3X	(4 and/or 5),6,9,10[W]
'Guinzam' (Guinevere®)	2.52	3X	(4 and/or 5),9
'Candy Mint'	2.53	3X	(4 and/or 5)
'Camzam'(Camelot®)	2.54	3X	(4 and/or 5)
'Prairie Maid'	2.57	3X	(4 and/or 5), 11[W]
'Kinarzam' (King Arthur™)	2.59	3X	(4 and/or 5),7,9
hupehensis (1998-205)	2.61	3X	6,9
'Cardinal'	2.62	3X	(4 and/or 5) [W]
sargentii (1998-243)	3.36	4X	13,14,15,16
'Mary Potter'	3.37	4X	13,15,16
'Strawberry Parfait'	3.46	4X	12,16
'Branzam' (Brandywine™)	3.74	4X	15
LSD 0.05	0.10		

[Z]Numbers in parentheses are accession numbers.

[Y]Values are means, n=3.

[X]Refer to Table 1 for pathway descriptions.

[W]Observed some histograms with multiple peaks (e.g., 2C,3C,4C), possibly representing endopolyploidization or polyembryony.