Variation in Ploidy Level Among Flowering Crabapples

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Nature of Work: Ploidy level refers to the number of complete sets of chromosomes that an organism contains. Plants with two sets of chromosomes are referred to as diploid (2*x*), three sets triploid (3*x*), and so on with tetraploid (4*x*), pentaploid (5*x*), hexaploid (6*x*), etc. Polyploidy (more than two sets of chromosomes) is common in plants and has played an important role in plant evolution (6). There are many opportunities for developing and utilizing polyploids in plant improvement programs including overcoming barriers to hybridization, restoring fertility in wide hybrids, developing sterile cultivars, enhancing flower size, increasing heterosis and vigor, and improving pest resistance and tolerance to environmental stresses (7). Although ploidy level can be determined by counting chromosomes, flow cytometry provides a reliable and much faster means for determination of nuclear DNA content and associated ploidy level (1, 3). The objective of this study was to determine the approximate genome size and estimated ploidy level of a diverse collection of flowering crapbapples.

Leaf samples were collected from flowering crabapples (*Malus* Mill spp.) growing in an established field plot arranged as a randomized complete block experimental design with three replications. Leaf samples were also collected from an individual tetraploid tree of *M. hupehensis* R3T3-SF with a known genome size of 2C = 3.46 pg (2) for validating our methodology. Approximately 0.5 cm^2 each of sample 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. A minimum of 5,000 nuclei was measured per sample. Genome sizes were calculated as nuclear DNA content for unreduced tissue (2C) as: 2C DNA content of sample = (mean fluorescence value of sample x 2C 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 (4).

Results and Discussion: Our method estimated the 2C DNA content for *Malus hupehensis* R3T3-SF to be 3.40 pg (Fig. 1), which was consistent with 3.46 pg reported by Benson et al. (2) when determined using laser flow cytometry and propidium iodide as a fluorochrome. Our methodology also resulted in little experimental error with LSD 0.05 values among cultivars of only 0.10. There was considerable variation in DNA content among the taxa surveyed (Table 1). Prior reports have indicated that DNA contents for *Malus* spp. ranged from 1.2 - 1.85 for diploid, 2.07 - 2.73 for triploid, and 2.80 - 3.75 pg/2C for tetraploid apples and

crabapples (2). Results from the taxa included in our survey were similar, with DNA contents ranging from 1.52 to 1.82 for diploids, 2.40 to 2.62 for triploids, and 3.36 to 3.74 pg/2C for tetraploids. Based on these ranges, we identified 43 diploid, 10 triploid, and 4 tetraploid crabapple taxa in this collection. Although 'Mary Potter' has been reported to be a triploid (5), we found our accession to be a tetraploid. In most cases, ploidy levels of these specific taxa have not been previously reported. However, variation in ploidy levels among *Malus* taxa has been well documented and there can be natural variation in ploidy levels among and within species (5), emphasizing the need for cultivar specific data. The large number of triploids found in this survey was somewhat surprising. Although most of these triploids produce fruit, they probably have little potential for breeding. Seeds from these trees may be the result of apomixis or may have low viability resulting from aneuploidy.

Significance to Industry: Information on ploidy levels of cultivars is extremely valuable for use in plant breeding programs. Fertility, crossability, and heritability of traits are all influenced by ploidy levels. This research provides information on ploidy levels for specific cultivars of flowering crabapples and will allow for more systematic and efficient progress in the development of improved flowering crabapples.

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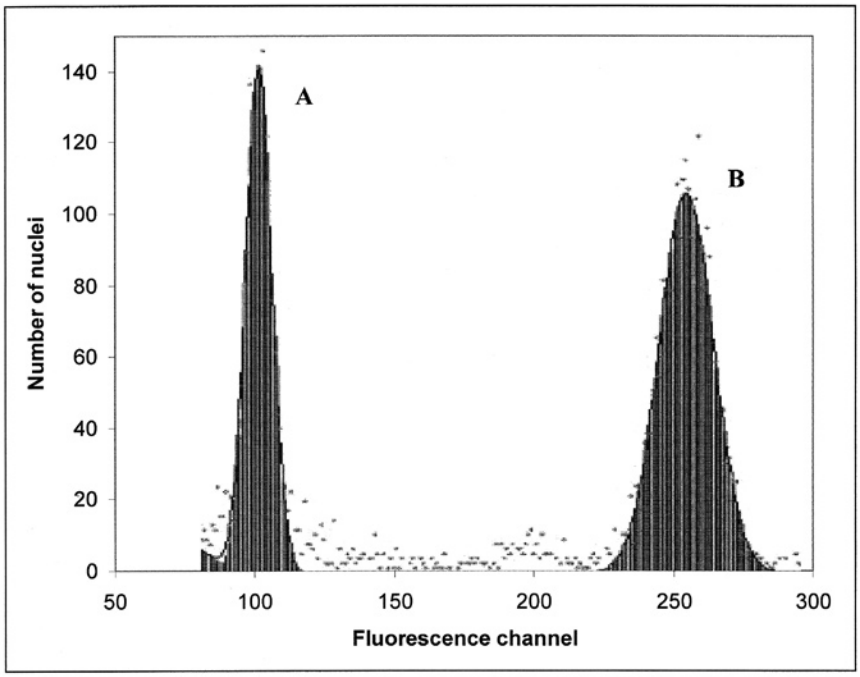

Figure 1. Histogram of relative DNA content of isolated nuclei for tetraploid *Malus hupehensis* R3T3-SF (A), with a mean fluorescence of 100.33, and *Pisum sativum* 'Ctirad' (B), with a mean fluorescence of 268.23.

Table 1. Approximate genome size and estimated ploidy level of *Malus* taxa determined from flow cytometry analysis of isolated nuclei.

Taxa	Approximate genome Size (pg)	Estimated ploidy level
tschonoskii (1998-242) [Z]	1.52 [Y]	2X
'Lanzam' (Lancelot®)	1.61	2X
'Red Splendor'	1.67	2X
'Molten Lava'	1.67	2X
'Sutyzam' (Sugar Tyme™)	1.67	2X
'Silver Dust'	1.68	2X
'Indian Summer'	1.68	2X
'Snow Drift'	1.68	2X
'JFS-KW5' (Royal Raindrops™)	1.68	2X
'Robinson'	1.69	2X
'Sinai Fire'	1.69	2X
'Centzam' (Centurion®)	1.70	2X
'Donald Wyman'	1.70	2X
'Prairifire'	1.70	2X
'Schmidtcutleaf' (Golden Raindrops™)	1.70	2X

sieboldii 'Calocarpa' (1998-238)	1.71	2X
'Canary'	1.71	2X
'Adams'	1.72	2X
'Professor Sprenger'	1.72	2X
'Morning Sun'	1.72	2X
'Pink Satin'	1.72	2X
'Hargozam' (Harvest gold™)	1.72	2X
floribunda (1998-199)	1.72	2X
'Glen Mills'	1.73	2X
'White Angel'	1.73	2X
'Sentinel'	1.73	2X
baccata 'Jackii' (1998-218)	1.73	2X
'Luwick'	1.74	2X
'Callaway'	1.74	2X
'Mazam' (Madonna™)	1.74	2X
'Dolgo'	1.74	2X
'Baskatong'	1.74	2X
'Liset'	1.74	2X
'Narragansett'	1.75	2X
'Louisa'	1.75	2X
'David'	1.75	2X
'Purple Prince'	1.75	2X
'Jewelberry'	1.76	2X
'Cinzam' (Cinderella®)	1.76	2X
'Radiant'	1.77	2X
'White Cascade'	1.78	2X
'Doubloons'	1.78	2X
'Ormiston Roy'	1.82	2X
'Pink Princess'	2.40	3X
'Adirondack'	2.48	3X
'Silver Moon'	2.51	3X
'Guinzam' (Guinevere®)	2.52	3X
'Candy Mint'	2.53	3X
'Camzam'(Camelot®)	2.54	3X
'Prarie Maid'	2.57	3X
'Kinarzam' (King Arthur™)	2.59	3X
hupehensis (1998-205)	2.61	3X
'Cardinal'	2.62	3X
sargentii (1998-243)	3.36	4X
'Mary Potter'	3.37	4X
'Strawberry Parfait'	3.46	4X
'Branzam' (Brandywine™)	3.74	4X
LSD $_{0.05}$	0.10	

[Z] Numbers in parentheses are accession numbers.

[Y] Values are means, n=3.