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Genome Size and Ploidy Levels of *Cercis* (Redbud) Species, Cultivars, and Botanical Varieties

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***Abstract. Cercis* is an ancient member of Fabaceae, often cultivated as an ornamental tree, and can be found in numerous regions around the world. Previous studies have reported *Cercis canadensis* as being diploid with $2n = 2x = 14$. However, there have been no further investigations into ploidy and genome size variation among *Cercis* taxa. A study was conducted to evaluate the relative genome size and ploidy levels of numerous species, cultivars, and botanical varieties of *Cercis*, representing taxa found in North America, Asia, and the Middle East. In addition, the genome size of *Bauhinia forficata*, a close relative of *Cercis*, was also determined. Genome size estimates (2C values) were determined by calculating the mean fluorescence of stained nuclei via flow cytometry. Propidium iodide was used as the staining agent and *Glycine max* was used as an internal standard for each taxon analyzed. Genome size estimates for all *Cercis* sampled ranged from 0.70 to 0.81 pg with an average size of 0.75 pg. The genome size of *B. forficata* was found to be smaller than any other *Bauhinia* sp. currently on record, with an average size of 0.87 pg. This study confirmed an initial estimation of the genome size of *Cercis chinensis* and found that floral buds of *Cercis* proved to be an excellent source of plant tissue for obtaining intact nuclei. All species, botanical varieties, and cultivars of *Cercis* surveyed for this study had remarkably similar genome sizes despite their wide range of distribution. This information can facilitate a better understanding of phylogenetic relationships within Cercideae and *Cercis* specifically.**

The genus *Cercis* L. (Fabaceae: Caesalpinoideae: Cercideae), also known as redbud, is a valuable commodity in the North American landscape industry and can be found growing in temperate environments across the globe. *Cercis* consists of ≈10 species (Davis et al., 2002; Fritsch et al., 2009), which can be found in North America (*C. canadensis* L., *Cercis occidentalis* Torr. ex A. Gray), Asia (*C. chinensis* Bunge, *Cercis chingii* Chun, *Cercis chuniana* P.F. Metcalf, *Cercis gigantea* W.C. Cheng & Keng f., *Cercis glabra* Pamp, *Cercis racemosa* Oliv., and *Cercis siliquastrum* L.), and the Middle East (*Cercis griffithii* Boiss.). Redbud is recognized for a variety of interesting morphological characteristics, many of which make them ideal ornamental specimens. Valuable insight into angiosperm evolution can be obtained through genetic surveys of this valuable landscape commodity.

Fabaceae, one of the most successful lineages of flowering plants (Legume Phylogeny Working Group et al., 2013) has long been the subject of genomics and genetic research. In particular, species like *Lotus japonicus* (Regel) K. Larsen and *Medicago truncatula* Gaertner have been adopted internationally as genetic models for legume-based research thanks to their model characteristics (Udvardi et al., 2005). Caesalpinoideae, a subfamily in which Cercideae resides, contains much of the evolutionary and genetic diversity found in all of Fabaceae. However, recent studies have focused on cultivated legume crops, all of which have diverged relatively recently (Young et al., 2003). This information covers only a fraction of the great diversity that can be found within Fabaceae (Doyle and Luckow, 2003) and could be further supplemented by studying a basal, non-nitrogen fixing member of Fabaceae such as *Cercis*.

Cercis, an ancient member of Caesalpinoideae, has fossil records that date back to the Eocene era (Jia and Manchester, 2014), and is therefore a prime candidate for a comprehensive study of genome size as it relates to legume systematics and taxonomy. Most aspects of legume biology, from ploidy number to floral diversity, can be further examined through the evolutionary relationships that exist among leguminous taxa (Young et al., 2003). This information could grant valuable insight into species evolution and provide potential breeding applications (Rounsaville and Ranney, 2010) in future *Cercis* hybridization projects.

Information derived from a genome survey of *Cercis* will be useful as it relates to a better understanding of the evolution of genome size and ploidy distribution within the legumes. Three cultivars of *Cercis* warrant particular interest in regard to ploidy variation. Traveller is a unique cultivar of *C. canadensis* var. *texensis* possessing both male and female sterility. The basis of this sterility is unknown, but potentially could be based on triploidy. Triploid plants often have reduced fertility or sterility. Likewise, 'Don Egolf' is a female sterile form of *C. chinensis* and will be investigated to determine if its sterility is due to triploidy. Finally, 'Tom Thumb' a diminutive sterile form of *C. canadensis* with extremely small leaves and flowers will be investigated to determine if its unique characters are potentially due to haploidy. Haploid plants have been shown to exhibit dwarfism in other woody species (Yahata et al., 2005). This survey will also contribute to the knowledge of the taxonomic relationship of *Bauhinia* to redbud. *Cercis* has been documented as having seven chromosome pairs with $2n = 2x = 14$ (Curtis, 1976; Goldblatt, 1981). *Bauhinia* is thought to be a tetraploid ($2n = 4x = 28$) relative of *Cercis* (Doyle and Luckow, 2003) with 14 chromosome pairs (Turner, 1956). As the closest living relative of redbud (Coskun and Parks, 2009), *Bauhinia* will serve as an interesting comparison with the relative DNA estimations of *Cercis* in this study.

Cercis chinensis possesses a relatively small genome size of 350 million bps (Mb) (De Mita et al., 2014), which corresponds with the phylogenetic position of significant antiquity (Zou et al., 2008) that *Cercis* occupies within Fabaceae. Except for *C. chinensis*, there are currently no other reports of genome size of *Cercis*. The objectives of this study were to examine the genome size of a comprehensive collection of species, botanical varieties, and cultivars of *Cercis.* Additionally, the genome size of 'Tom Thumb', demonstrating morphological characters suggesting haploidy, and of 'Traveller' and 'Don Egolf', sterile cultivars suggesting triploidy, will be examined.

Materials and Methods

Plant material. For the purposes of this study, nine species of *Cercis* and one species of *Bauhinia* (*B. forficata* Link) were surveyed for relative DNA content. A total of 30 taxa (Table 1) were surveyed, including three botanical varieties of *Cercis*: *C. canadensis* var. *canadensis*, *C. canadensis* var. *mexicana* (Rose) M. Hopkins, and *C. canadensis* var. *texensis* (S. Watson) M. Hopkins. This survey also included a number of cultivars and hybrids that exhibit the full spectrum of morphological variation found in *Cercis.* *Glycine max* L. (2C DNA = 2.25 pg) was

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Table 1. Genome size and estimated ploidy levels of *Cercis* species, cultivars, and botanical varieties.

Taxa	Source[z]—accession no.	2C genome size (pg)[y]	Mean 1C genome size (pg) by species	Estimated ploidy level (*x*)
Cercis canadensis	JCRA—050030	0.76 ± 0.01	0.37 ± 0.01	2*n* = 2*x*
C. canadensis 'Ace of Hearts'	JCRA—040037	0.72 ± 0.00		2*n* = 2*x*
C. canadensis 'Appalachia Red'	JCRA—100164	0.77 ± 0.01		2*n* = 2*x*
C. canadensis 'Dwarf White'	JCRA—020083	0.76 ± 0.01		2*n* = 2*x*
C. canadensis 'Flame'	JCRA—990614	0.71 ± 0.01		2*n* = 2*x*
C. canadensis 'Floating Clouds'	JCRA—050027	0.74 ± 0.01		2*n* = 2*x*
C. canadensis 'Forest Pansy'	JCRA—980909	0.77 ± 0.01		2*n* = 2*x*
C. canadensis 'Greswan'	JCRA—100497	0.72 ± 0.01		2*n* = 2*x*
C. canadensis 'Hearts of Gold'	JCRA—040050	0.73 ± 0.00		2*n* = 2*x*
C. canadensis 'JN2'	JCRA—100498	0.75 ± 0.01		2*n* = 2*x*
C. canadensis 'Little Woody'	JCRA—040036	0.78 ± 0.03		2*n* = 2*x*
C. canadensis 'Ruby Falls'	JCRA—100167	0.74 ± 0.02		2*n* = 2*x*
C. canadensis 'Silver Cloud'	JCRA—030265	0.74 ± 0.01		2*n* = 2*x*
C. canadensis 'Tom Thumb'	JCRA—050031	0.76 ± 0.01		2*n* = 2*x*
C. canadensis var. *mexicana*	JCRA – xx041	0.72 ± 0.01		2*n* = 2*x*
C. canadensis var. *mexicana* NC4	Unaccessioned JCRA selection	0.78 ± 0.01		2*n* = 2*x*
C. canadensis var. *texensis* 'Oklahoma'	JCRA—090011	0.77 ± 0.02		2*n* = 2*x*
C. canadensis var. texensis 'Traveller'	JCRA—960536	0.74 ± 0.01		2*n* = 2*x*
C. canadensis [Texensis Group] 'Merlot'	JCRA—090079	0.72 ± 0.02		2*n* = 2*x*
C. chinensis	JCRA—xx044	0.76 ± 0.01	0.37 ± 0.00	2*n* = 2*x*
C. chinensis 'Don Egolf'	JCRA—050037	0.74 ± 0.01		2*n* = 2*x*
C. chinensis 'Shirobana'	JCRA—020089	0.74 ± 0.01		2*n* = 2*x*
C. chingii	JCRA—020086	0.81 ± 0.01	0.40 ± 0.00	2*n* = 2*x*
C. gigantea	JCRA—020079	0.81 ± 0.00	0.41 ± 0.00	2*n* = 2*x*
C. glabra	JCRA—920545	0.75 ± 0.01	0.38 ± 0.00	2*n* = 2*x*
C. glabra 'Celestial Plum'	JCRA—090316	0.77 ± 0.00		2*n* = 2*x*
C. griffithii	CRKA	0.70 ± 0.01	0.35 ± 0.00	2*n* = 2*x*
C. occidentalis	CRKA	0.74 ± 0.01	0.37 ± 0.00	2*n* = 2*x*
C. racemosa	JCRA—080062	0.78 ± 0.01	0.39 ± 0.01	2*n* = 2*x*
C. siliquastrum	USNA—118kj 37350	0.77 ± 0.02	0.38 ± 0.01	2*n* = 2*x*
Bauhinia florficata	PDN	0.87 ± 0.01	0.43 ± 0.00	2*n* = 2*x*

[z]JCRA = JC Raulston Arboretum, Raleigh, NC; USNA = United States National Arboretum, Washington, DC; CRKA = Charles R. Keith Arboretum, Chapel Hill, NC; PDN = Plant Delights Nursery, Raleigh, NC.

[y]Genome size for each taxon was determined from two independent analyses conducted in Jan. 2014 and Jan. 2015. At each sampling date, two subsamples were prepared for each taxon analyzed. Values shown represent the mean and the SE of the mean of the two sampling dates.

used as the internal standard for all taxa surveyed.

Sample preparation. Newly expanded leaf tissue of *Cercis* proved recalcitrant to obtaining adequate quantities of intact nuclei. Therefore, a protocol modified from Dolezel et al. (2007) was employed for the assay procedure. Floral buds were used as plant tissue for all *Cercis* sampled. Plant tissue was obtained from the U.S. National Arboretum (Washington, DC), the JC Raulston Arboretum (Raleigh, NC), and the Charles R. Keith Arboretum (Chapel Hill, NC). All taxa were assayed once in Jan. 2014 and again in Jan. 2015.

Expanding floral buds were harvested from bud sticks collected in late January and forced for ≈10–14 d under greenhouse conditions. For each sample, about six to eight floral buds were collected and placed into a 60 × 15 mm petri dish containing damp filter paper. Buds were descaled and refrigerated at 4 °C before sample preparation. New but fully expanded leaves obtained from container-grown *G. max* plants grown under greenhouse conditions were used as the internal standard for each sample. Sample preparation required about three floral buds from the *Cercis* sample to be chopped with 1 cm^2 of leaf tissue from the *G. max* standard. Combined plant tissues were chopped finely with a double-edged razor blade (Personna stainless steel double edge prep blades, Edgewell Personal Care, Shelton, CT) in a 60 × 15 mm petri dish. Chopped plant material was gently agitated in 500 µL of nuclei extraction buffer (Cystain® PI Absolute P, Sysmex, Germany) and after ≈60 s of incubation, suspension was poured through a 50-µm nylon mesh filter into a small, polystyrene test tube. The resulting nuclei suspension was then stained using 1500 µL of propidium iodide staining solution (Cystain® PI Absolute P), prepared by combining 2 mL staining buffer, 12 µL propidium iodide, and 6 µL of RNase per sample. Two subsamples were prepared for each taxon analyzed. Stained nuclei suspensions were refrigerated at 4 °C for 1 h before being analyzed via flow cytometry. All samples were completely randomized before analysis.

Flow cytometry. Holoploid genome size estimates were determined by measuring the relative fluorescence of stained nuclei via flow cytometry. Analysis of each sample was conducted on a BD LSR II flow cytometer (Becton-Dickson Biosciences, San Jose, CA) operating with a 20-mW argon laser (excitation = 488 nm). Histograms that display mean fluorescence values were compiled until a minimum cell count of 5000 was achieved for each sample. Cell counts were acquired using BD FACSDiva software (Becton-Dickson Biosciences) and analyzed using FCS Express (De Novo Software, Los Angeles, CA). Holoploid genome size estimates for each sample were calculated as: 2C = (mean fluorescence of sample ÷ mean fluorescence of standard) × (2C value of standard). Chromosome number of diploid *C. canadensis* has been documented as 2*n* = 2*x* = 14 (Curtis, 1976; Goldblatt, 1981) and was compared with genome size estimates and used to infer ploidy of taxa sampled.

Results and Discussion

Floral buds proved to be an excellent source for obtaining intact nuclei (Fig. 1) and proved to be a more viable option than leaf tissue among all *Cercis* sampled. Results of flow cytometric analysis show that holoploid genome size estimates among *Cercis* taxa were relatively small and highly conserved across species, with sizes ranging from 0.70 to 0.81 pg (Table 1) with a mean of 0.75 pg. Comparatively, the 2C genome size estimates of *Lotus japonicus* and *M. truncatula* (both model organisms) have each been confirmed to be 0.95 pg (Bennett and Leitch, 2011). Previous studies that used foliar tissue estimated the monoploid (1Cx) genome size of *C. chinensis* to be 350 Mb (0.36 pg). This study obtained similar values by using floral bud tissue, with 1Cx genome size estimates for *C. chinensis* ranging from 356.97 to 381.42 Mb (0.37–0.39 pg) with an average of 371.64 Mb (0.38 pg).

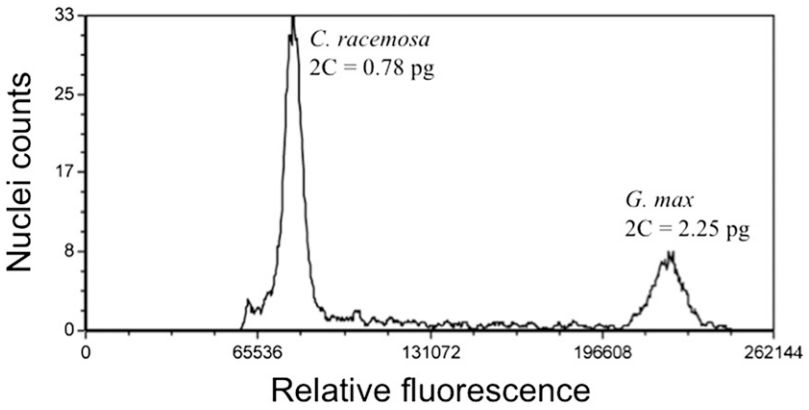

Fig. 1. Histogram displaying mean fluorescence of stained nuclei, obtained from floral buds of *Cercis racemosa* Olive and leaf tissue of *Glycine max* L.

A query of the Kew Royal Botanic Garden DNA C-values database (Bennett and Leitch, 2012) shows that *Cercis* possesses the smallest 2C genome size (0.75 pg on average) of all reported members of Fabaceae, reflecting its antique phylogenetic position. However, the diminutive nature and lack of variability in genome size among all *Cercis* sp. is surprising given the long temporal and geographic isolation the taxa have experienced. Genome size and variability is thought to increase in size as speciation occurs. Variation in genome size is especially noticeable in species with extensive geographic distribution, high degrees of morphological differentiation, and several subspecific categories (Huang et al., 2013). Interestingly, *Cercis* sp. meet all of these criteria but have not shown a significant increase in genome size or polyploidization events, as would be expected of such an ancient genus.

The high degree of morphological variation found in *Cercis*, and the existence of sterility, suggest that several cultivars of particular interest could have arisen from variations in ploidy. However, three specific cultivars subject to genome size estimation, Traveller (sterile), Don Egolf (sterile), and Tom Thumb (very small leaves and flowers) were diploid. The lack of variability in ploidy among *Cercis* taxa is informative, as ancient polyploidization events have been associated with increases in plant diversity in Fabaceae (Doyle et al., 2012; Schranz et al., 2012; Soltis and Burleigh, 2009; Soltis et al., 2014).

Contrary to previous studies that have reported *Bauhinia* being a tetraploid relative of *Cercis*, 2C estimates of *B. forficata* place its genome size at 0.87 pg (Table 1). If *B. forficata* was tetraploid, one would expect its 2C genome size to approach 1.4 pg, as is the case with *Bauhinia monandra* Kurz (Ohri et al., 2004). There is evidence of small ancestral genome sizes among angiosperms (Leitch et al., 1998), and it could be that *B. forficata* is a more ancestral member of the genus. It is also possible that most *Bauhinia* sp. are tetraploid, and that *B. forficata* experienced a reduction in chromosome number. Every major evolutionary line of legume seems to have experienced some degree of descending aneuploidy (Goldblatt, 1981), which could explain the small genome size of *B. forficata*, relative to the rest of the genus. No other studies have reported the genome size or chromosome number of *B. forficata* and as such, further research into its relationship with other members of Cercideae could prove to be informative.

Conclusions

Our data confirmed an initial report of the genome size of *C. chinensis*. This study revealed that all *Cercis* taxa surveyed had remarkably similar genome sizes despite their wide range of phenotypic diversity and wide geographic distribution. Furthermore, all taxa surveyed proved to be diploid, despite our initial hypotheses of potential haploidy in *C. canadensis* 'Tom Thumb', and potential triploidy in *C. canadensis* var. *texensis* 'Traveller' and *C. chinensis* 'Don Egolf'. Estimates of 2C genome size among all *Cercis* surveyed ranged from 0.70 to 0.81 pg with an average size of 0.75 pg. Model legumes such as *M. truncatula* and *L. japonicus*, both nitrogen fixing species, are known for having "compact" genome sizes, with 2C values of ≈929 Mbp or 0.95 pg (Young et al., 2003). *Cercis* is an ancient genus possessing a smaller genome size than both *M. truncatula and L. japonicus,* supporting the hypothesis that a whole genome doubling event occurred shortly after the origin of rhizobial symbiosis in Fabaceae but did not affect more basal legume lineages of Caesalpinoideae (De Mita et al., 2014). *Cercis* is widely regarded as an out-group of Fabaceae due to its inability to fix nitrogen. The symbiosis that developed between plants and the nitrogen-fixing bacteria known as rhizobia occurred ≈60 million years ago and could be one of the contributing factors that gave rise to more than 19,000 species of legume (De Mita et al., 2014; Doyle, 1998; Doyle et al., 2012; Legume Phylogeny Working Group et al., 2013). As a basal member of the legumes, *Cercis* was likely external to the evolutionary event that led to the symbiotic relationship with rhizobial bacteria (De Mita et al., 2014). As such, *Cercis* could prove invaluable in future studies of legume speciation.

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