ABSTRACT

ROBERTS, DAVID JONATHAN. Genome Size Determination, and Inheritance and Allelism of Morphological Traits in *Cercis* (Under the direction of Dr. Dennis J. Werner and Dr. Thomas G. Ranney).

Cercis is an ancient member of Fabaceae, often cultivated as an ornamental tree, and can be found growing in numerous regions around the world. Previous studies have reported *Cercis* 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. Relative genome size estimates (2C values) were determined by calculating the mean fluorescence of stained nuclei via flow cytometry. Floral buds of *Cercis* proved to be an excellent source of plant tissue for obtaining intact nuclei. 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 a prior estimation of genome size of *C. chinensis*. All species, botanical varieties, and cultivars of *Cercis* surveyed for this study had remarkably similar genome sizes despite their wide range of distribution and temporal isolation. This information can facilitate a better understanding of phylogenetic relationships within Cercideae and provide valuable insight into legume speciation.

Inheritance of purple, gold, and variegated foliage types, weeping architecture, and double flower was explored in F_1, F_2, and backcross families resulting from controlled hybridization of eastern redbud (*Cercis canadensis* L.). Potential allelic relationships were explored when possible. Inheritance analysis in families derived from controlled hybridization of 'Covey' (green leaf) and 'Forest Pansy' (purple leaf) suggest that purple leaf color and weeping architecture are both controlled by single recessive genes, for which the symbols *pl1* and *wp1* are proposed, respectively. Inheritance of gold leaf was explored in families of 'Covey' (green leaf) x 'Hearts of Gold' (gold leaf). Interpretation of inheritance of gold leaf in these families was confounded by the recovery of a leaf color phenotype in the F_2 family unlike either parent. However data suggested the action of a single locus controlling gold leaf color in 'Hearts of Gold', and that instability of gold leaf expression may be based on transposable element activity. Segregation of gold leaf in the F_2 families of 'Texas White' [green leaf (*C. canadensis* var. *texensis*)] x 'JN2' [gold leaf (The Rising Sun®)]did not fit a Mendelian ratio. Analysis of progeny of 'Silver Cloud' and 'Floating Clouds' (both showing white/green leaf variegation) with non-variegated cultivars demonstrated that variegation in 'Silver Cloud' is controlled by a single recessive nuclear gene, while variegation in 'Floating Clouds' is controlled by cytoplasmic factors. The symbol *var1* is proposed for the gene controlling variegation in 'Silver Cloud'. Double flower in progeny derived from 'Flame' (double flower) suggested that double flower is dominant to single flower, and that 'Flame' is heterozygous at the double-flower locus, for which the symbol *Df1* is proposed. Allelism studies showed that the gene controlling purple leaf in 'Forest Pansy' is allelic to the purple leaf gene in 'Greswan' and that the gene controlling weeping phenotype in 'Traveller' (*C. canadensis* var. *texensis*) is non-allelic to the weeping

gene found in 'Covey'. Allelism of the gold leaf trait in 'Hearts of Gold' and 'JN2' was investigated, but no clear conclusions regarding allelism could be made due to recovery of leaf color phenotypes unlike either parent.

Genome Size Determination, and Inheritance and Allelism of Morphological Traits in *Cercis*

By
David Jonathan Roberts

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Horticultural Science

Raleigh, North Carolina
2015

APPROVED BY:

Dr. Dennis J. Werner Co-chair	Dr. Thomas G. Ranney Co-chair

Dr. Susana Milla-Lewis
Committee

DEDICATION

In memory of my loving father, Ronald G. Roberts and for my wonderful parents, Shirley A. Wells and Michael H. Wells.

BIOGRAPHY

David Jonathan Roberts was born in Buffalo, NY to the loving parents of Ronald and Shirley Roberts. After several years, Ron and Shirley moved their family to Boone, NC so that David and his younger brother Jon could have a safe and natural environment in which they could spend their childhood. With the mountains of western North Carolina as his playground, David developed a love for nature early in life. After graduating from high school, David moved to Asheville, so that he could pursue a degree in computer programming. However, constantly gazing out the window of his classrooms, he eventually realized that a career spent behind computer monitors, beneath fluorescent lights, was not the kind of life he wanted. David took a hiatus from school so that he could work full time and gain a better understanding of what his career path should be. It was not until 2004 when he lost his father that David realized he should pursue a career that would make him happy rather than wealthy. With the help of friends and family, David soon realized that horticulture was his best possible option for such a career. Fortunately, Asheville proved to be an ideal environment for nurturing his budding passion.

A love for bonsai drew David to the NC Arboretum where he spent his free time volunteering and was eventually employed by the curator of the bonsai collection, Arthur Joura. Arthur taught David, not only the artistic aspects of bonsai, but the underlying horticulture that was essential for a tree's survival. His time spent at the arboretum helped David understand that, in order to pursue a career in horticulture, he would need to return to school to further his knowledge and experience. Taking out several loans and working part time to pay for tuition, David registered for classes at Haywood Community College and was

soon enrolled in his first courses in horticulture. After several semesters, David moved to Raleigh to pursue his ultimate goal, a master's degree from the horticulture department of North Carolina State University.

NC State proved to be fertile ground for a rapidly growing passion in horticulture and applied plant sciences. The faculty and staff of the undergraduate department of horticulture provided motivation and inspiration for developing plant scientists and instantly made David feel at home. It was during a course in herbaceous perennials that David encountered Dr. Dennis Werner and developed an interest in plant breeding. Thanks to Dr. Werner's tutelage and guidance, David was soon making controlled hybridizations with redbuds and his interest quickly developed into passion. Dr. Werner employed David during the summer months and later encouraged him to apply for an internship with Dr. Tom Ranney at the Mountain Crop Improvement Lab in Mills River, NC. After being accepted, he found himself back in western NC, this time as part of an elite team of plant breeding professionals and graduate students. During his months at the MCIL, it became apparent that a career in plant breeding was no longer just possible but highly desirable.

The semester before completing his undergraduate work, David was presented with an opportunity to pursue a master's degree in horticultural science. Under the direction of Dr. Werner, David learned to think, not just as a plant breeder, but as a scientist. Making crosses, gathering seed and growing on progeny became more than a means of generating exciting new cultivars, it became a tool that future scientists and plantsman could utilize for generations to come.

As a graduate student, David has given lectures in plant breeding and propagation, acted as vice president for the horticulture society of Pi Alpha Xi, served as a TA for plant propagation labs and presented his research at plant breeding conferences. David has learned techniques and developed skills that will serve him for the rest of his life and allow him to acquire a career in the field he loves most. David's passion for horticulture, and plant breeding specifically, has grown exponentially, thanks to his "dream team" of advisors and the many friends and family who have helped support him over the years.

ACKNOWLEDGEMENTS

I feel it would take an entire thesis to adequately thank all of the people who have helped me get to this point in my career. As such, I will try my best to acknowledge all those who have had an impact on my life and I ask forgiveness of those whom I may have unintentionally omitted. Of course, my amazing family must be acknowledged for providing me with a safe and loving environment in which to grow. My late father Ron, who taught me that all work is honorable if it's done well, continues to motivate me to this day. He instilled within me a work ethic that I will carry for the rest of my life and his passing inspired me to pursue a life that he would be proud of. My mother Shirley, who always provides a silver lining whenever I need it, encourages me to pursue any and everything my heart desires. Her love and perpetual positivity helped carry me through some of my darkest days. My second father Michael, whose rock solid foundation of love and support never leave me wanting for encouragement. His constant confidence has always reassured me that I'm doing the right thing. My brother, my best friend and my partner in crime, Jon. We have been through so much together that it's hard for me to imagine who I would be, were it not for you. Your strength and fortitude are an inspiration and I'm constantly amazed that my younger brother can often be more mature than me. I have an amazing family and I love you all so much.

The friends I have acquired over the years have been an integral part of maintaining my sanity through various trials and tribulations. Antonio Del Toro, you helped me get back into "school mode" when I was working full time and encouraged me to pursue a degree sooner rather than later. Rich Delalio, your kindness and compassion have helped me through some of the most difficult times of my life. My fellow plant breeding buddies Kim

and Jason Lattier, you guys are both truly inspirational and have been some of the best role models I've ever had. Keith Lukowski, Sarah Leach-Smith, Tom and Camie Gargano have been my horticultural soul mates for as long as I've known them. Ted Yap, Jeremy Mahachek, Dominic Gilooly and Whitney Phillips have been my grad school compatriots, suffering and triumphing with me through the many challenges grad school presents. And of course, my incredibly patient and utterly amazing girlfriend Amila Chapman. You were one of the first people to encourage me to pursue a career in horticulture. Your academic super powers inspired me to become the best student I could be. Thank you for always being there for me, and for always believing in me. I've always loved plants, but your calm confidence is just one of the many reasons I love you. The love that my family and friends have provided has nourished me when I've needed it most, and I am proud to have them in my life, and to be a part of theirs.

The incredible staff of the North Carolina Arboretum in Asheville, provided my first hands on experience in horticulture, and my life hasn't been the same since. June Jolly, whose greenhouse left me in awe after my first visit, taught me the value of cleanliness and integrated pest management in closed environments. Arthur Joura, an artist and true plantsman, taught me that nature must be respected, and that only through knowledge and understanding can that respect be properly paid. Arthur, you took a kid who knew practically nothing about plants and inspired him to pursue a career with them. You helped set the trajectory of the path I'm on today and I can't thank you enough.

The faculty and staff of Haywood Community College, who helped me realize that school can actually be fun if one enjoys the topic of study. George Thomas, John Sherman

and Milton "Buddy" Tignor helped me take my love of plants to the next level. These wonderful instructors both encouraged and helped facilitate my move to Raleigh and my transfer to NC State.

The faculty and staff of North Carolina State University, who made me the horticulturist I am today. NC State not only provided me with a multitude of opportunities and connections but with scholarships that helped lighten the financial burden associated with attending one of the top universities in the nation. I have never before been part of such an amazing institution and I'm exceedingly proud to call myself an alumni of such a prestigious school. Dr. Brian Jackson not only helped me transfer to NC State but served as a terrific undergraduate advisor, constantly challenging me to take classes that would best serve me in the future. Dr. Chad Jordan of the plant biology department showed me that plant anatomy and physiology could not only be fun and interesting, but truly exciting as well. Sarah Schuett, manager of NC State's Flow Cytometry and Cell Sorting facility, provided unparalleled support and technical assistance for my genome survey. Sarah's expertise with cell sorting and analysis was utterly crucial and always delivered with kindness and a smile. Dr. Julia Kornegay, the director of Graduate Programs and her assistant Rachel McLaughlin, always kept me on track and disseminated crucial information in a timely and efficient manner.

Diane Mays, the teaching technician and supervisor for NC State's greenhouse conservatories, provided my first internship experience and supplied a wealth of knowledge pertaining to tropical plant species. Diane and I later worked together to establish the position of vice president of specialty plants for the horticulture society Pi Alpha Xi and the

time and energy she has invested in that position is unprecedented. Diane, you are truly one of the kindest people I have ever encountered and your knowledge of tropical, sub-tropical and xeric flora is simply astounding. Thank you for always lending an open ear and for being such a good friend.

Dr. Susana Milla-Lewis of the crop science department showed me that genetics wasn't nearly as scary and intimidating as I had imagined. The ease with which she explained complex topics gave me the confidence to tackle advanced classes in biology, chemistry and genetics. Susana, the advice and council you have provided over the years has been invaluable and I am beyond grateful to have such an amazing person as a part of my committee, and as a friend.

Dr. Tom Ranney and his all-star cast of plant breeders and technicians at the Mountain Crop Improvement Lab, made me a part of their incredible team and provided my first research-based experience. Dr. Darren Touchell and Jeremy Smith taught me the intricacies of tissue culture, and helped nurture my appreciation for high quality hops. Nathan Lynch taught me how to create cell suspensions and use a flow cytometer, and made it look as easy as sticking a cutting. Joel Mowrey and Tom Eaker showed me how to propagate and care for nursery crops on a mass scale and allowed me and my friend Kim to reinvigorate the research station's arboretum. Dr. Tom Ranney is a truly incredible plant breeder, scientist and co-chair of my committee. Tom allowed me to structure my first scientific experiment and granted me access to some of his research materials in order to do so. This was an experience unlike any I had been a part of before, and it cemented my love for plant-based breeding, science and research. Tom, your program is truly incredible and I hope that

someday, I too can find a similar place among such intelligent and professional individuals. The kindness, advice and guidance you and your team have provided over the years have been crucial for my development as a plant breeder.

Dr. Dennis Werner, co-chair of my committee and major advisor in matters of both life and academia. Once again, I feel I could dedicate an entire chapter to how much Dr. Werner has influenced my life and career over the few short years we have known each other. My first encounter with Dr. Werner was in a course he taught entitled Gardening with Herbaceous Perennials. This sounded like a fun class when I signed up for it, but I was unprepared for how much practical science and applied horticulture was built into the course. Dr. Werner discussed a wide range of topics, from alkaloid metabolism to eliminating apical dominance in agaves. However, it was when he described the process that generated the weeping, purple leaf redbud known as 'Ruby Falls', that my mind was truly blown. The sheer novelty and seeming ease with which he combined two desirable traits into a single organism was beyond exciting. I had just been introduced to ornamental plant breeding and my life would never be the same. Dr. Werner employed me in both his garden and his greenhouse and was soon advising me in all matters related to my horticultural future. He encouraged me to pursue internships and scholarships. When the time came to take my education to the next level, Dr. Werner offered to act as my advisor for a master's degree in horticultural science. Being a scientific neophyte, I soon found the balance of class work and research required of a grad student to be challenging. However, Dr. Werner addressed each issue I presented with patience and expertly articulated insight. Whether driving to the Sandhills of NC or flying into Nashville TN, Dr. Werner has always been open, honest and

receptive to any question I asked of him. As a part of his ornamental breeding program, I've learned about a wide range of topics that range from ploidy manipulation in banana to transposable element activity in redbud. He has taught me how to create advanced plant hybrids and shown me that determining the heritability of certain traits can further scientific research. I know that everything he has done for me has been an effort to make me a better scientist and human being. Dr. Werner, I am eternally grateful for everything you have done for me and I can only hope to someday pay forward all of the kindness, patience and time you have invested in me.

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Chapter 1

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

Genome size and ploidy levels of *Cercis* (redbud) species, cultivars and botanical varieties

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This research was funded by the NC Agricultural Research Service, J. Frank Schmidt Family Charitable Foundation, and the North Carolina Nursery and Landscape Association. Technical assistance provided by Dr. Tom Ranney, Nathan Lynch, Sarah Seschuet, Kim Shearer-Lattier and Jason Lattier is gratefully acknowledged. Special thanks to Dr. Richard Olsen and David Kidwell-Slak (United States National Arboretum) for providing samples of *Cercis siliquastrum* and to Dr. Charles R. Keith for providing samples of *C. siliquastrum, C. occidentalis* and *C. griffithii.*

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Subject Category: Breeding, cultivars, varieties and germplasm resources

Genome size and ploidy levels of *Cercis* (redbud) species, cultivars and botanical varieties

Additional index words: DNA content, flow cytometry, plant breeding, systematics, taxonomy.

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 utilized 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 *C. 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.

Introduction

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 approximately 10 species[1,2] that can be found in N. America (*C. canadensis* L., *C. occidentalis* Torr. ex A. Gray), Asia (*C. chinensis* Bunge, *C. chingii* Chun, *C. chuniana* P.F. Metcalf, *C. gigantea* W.C. Cheng & Keng f., *C. glabra* Pamp, *C. racemosa* Oliv., *C. siliquastrum* L.) and the Middle East (*C. griffithii* Boiss.). Rebud is often 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 has long been the subject of genomics and genetic research.[3] In particular, species like *Lotus japonicas* (Regel) K. Larsen and *Medicago truncatula* Gaertner have been adopted internationally as genetic models for legume based research thanks to their model characteristics.[4] 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.[5] This information covers only a fraction of the great diversity that can be found within Fabaceae and could be further supplemented by studying a basal, non-nitrogen fixing member of Fabaceae such as *Cercis*.[6]

Cercis, an ancient member of Caesalpinoideae, has fossil records that date back to the Eocene era, and is therefore a prime candidate for a comprehensive study of genome size as it relates to legume systematics and taxonomy.[7] Most aspects of legume biology, from ploidy number to floral diversity, can be further examined through the evolutionary relationships that exist among leguminous taxa.[5] This information could grant valuable insight into species evolution and provide potential breeding applications in future *Cercis* hybridization projects.[8]

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 regards 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. Lastly, '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.[9] This survey will also contribute to the knowledge of the taxonomic relationship of *Bauhinia* to redbud. *Cercis* has been documented as having 7 chromosome

pairs with $2n=2x=14$.[10,11] *Bauhinia* is thought to be a tetraploid ($2n=4x=28$) relative of *Cercis*[6] with 14 chromosome pairs.[12] As the closest living relative of redbud,[13] *Bauhinia* will serve as a control for the relative DNA estimations of *Cercis* samples.

C. chinensis possesses a relatively small genome size of 350 million base pairs[14] which corresponds with the phylogenetic position of significant antiquity that *Cercis* occupies within Fabaceae.[15] Except for *C. chinensis*, there are currently no other reports of genome size of *Cercis*.

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 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 Doleźel, Greilhuber and Suda was employed for the assay procedure.[16] Floral buds were utilized as plant tissue for all *Cercis* sampled. Plant tissue was obtained from the U.S. National Arboretum (Washington D.C.), the JC Raulston Arboretum (Raleigh, N.C.), the Charles R. Keith Arboretum (Chapel Hill, N.C.) and from research plots at the Sandhills Research

Station (Jackson Springs, N.C.). All taxa were assayed once in January 2014 and again in January 2015.

Expanding floral buds were harvested from bud sticks collected in January and forced for about 10-14 days under greenhouse conditions. For each sample, approximately 6-8 floral buds were collected and placed into a 60 x 15 mm petri dish containing damp filter paper. Buds were descaled and refrigerated at 4°C prior to 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 approximately three floral buds from the *Cercis* sample to be co-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, 6 Research Drive, Shelton, Conn.) in a 60 x 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 approximately 60 seconds 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 1,500 µL of propidium iodide staining solution (Cystain® PI Absolute P, Sysmex, Germany), 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 one hour before being analyzed via flow cytometry. All samples were completely randomized prior to analysis.

Flow Cytometry. Genome size estimates (2C) 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, Calif.) operating with a 20-mW argon laser (excitation=488 nm). Histograms that display mean fluorescence values were compiled until a minimum cell count of 5,000 was achieved for each sample. Cell counts were acquired using BD FACSDiva software (Becton-Dickson Biosciences, San Jose, Calif.) and analyzed using FCSExpress (De Novo Software, Los Angeles, Calif.). Holoploid, 2C 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 $2n=2x=14$[10,11] 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 (Figure 1) and proved to be a more viable option than leaf tissue among all *Cercis* sampled. Results of flow cytometric analysis show that holoploid, 2C genome size estimates among *Cercis* taxa were relatively small and highly conserved across species, with sizes ranging from 0.70 pg to 0.81 pg (Table 1) with a mean of 0.75 pg. Comparatively, the 2C genome size estimates of *L. japonicus* and *M. truncatula,* (both model organisms) have each been confirmed to be 0.95 pg.[17] Previous studies that utilized foliar tissue estimated the monoploid 1C genome size of *C. chinensis* to be 350 Mbp (0.36 pg). This study obtained similar values by utilizing floral bud tissue, with monoploid 1C genome size estimates for *C. chinensis* ranging from 356.97 – 381.42 Mbp (0.37 – 0.39 pg) with an average of 371.64 Mbp (0.38 pg).

A query of the Kew Royal Botanic Garden DNA C-values database[18] 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 is thought to increase in size and variability as speciation occurs. Variation in genome size is especially noticeable in species with extensive geographic distribution, high degrees of morphological differentiation and several sub-specific categories.[19] 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.[20,21,22,23]

Contrary to previous studies that have reported *Bauhinia* being a tetraploid relative of *Cercis* , 2C estimates of *B. forficata* place it's 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 *B. monandra* Kurz.[24] There is evidence of small ancestral genome sizes among angiosperms[25] 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,[11] 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 member 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 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 *Medicago truncatula* and *Lotus japonicus,* both nitrogen fixing species, are known for having "compact" genome sizes, with 2C values of approximately 929 Mbp or 0.95 pg.[5] *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.[14] *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 approximately 60 million years ago and could be one of the contributing factors that gave rise to more than 19,000 species of legume.[26,22,3,14] As a basal member of the legumes, *Cercis* was likely external to the evolutionary event that led to the symbiotic relationship with rhizobial bacteria.[14] As such, *Cercis* could prove invaluable in future studies of legume speciation.

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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)	Mean 1C genome size (pg) by species	Est. ploidy level (*x*)
C. 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	Sandhills	0.78 ± 0.01		2*n*=2*x*

Table 1 Continued.

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. gigantea	Sandhills / JCRA - 020079	0.81 ± 0.00	0.41 ± 0.00	2*n*=2*x*
C. glabra	Sandhills / 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. siliquastrum	CRKA / USNA – 118kj 37350	0.74 ± 0.02	0.37 ± 0.01	2*n*=2*x*
C. chingii	JCRA - 020086	0.81 ± 0.01	0.40 ± 0.00	2*n*=2*x*
C. racemosa	JCRA - 080062	0.78 ± 0.01	0.39 ± 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; Sandhills = Sandhills Research Station, Jackson Springs, NC; USNA = United States National Arboretum, Washington DC; CRKA = Charles R. Keith Arboretum, Chapel Hill, NC; PDN = Plant Delights Nursery, Raleigh, NC.

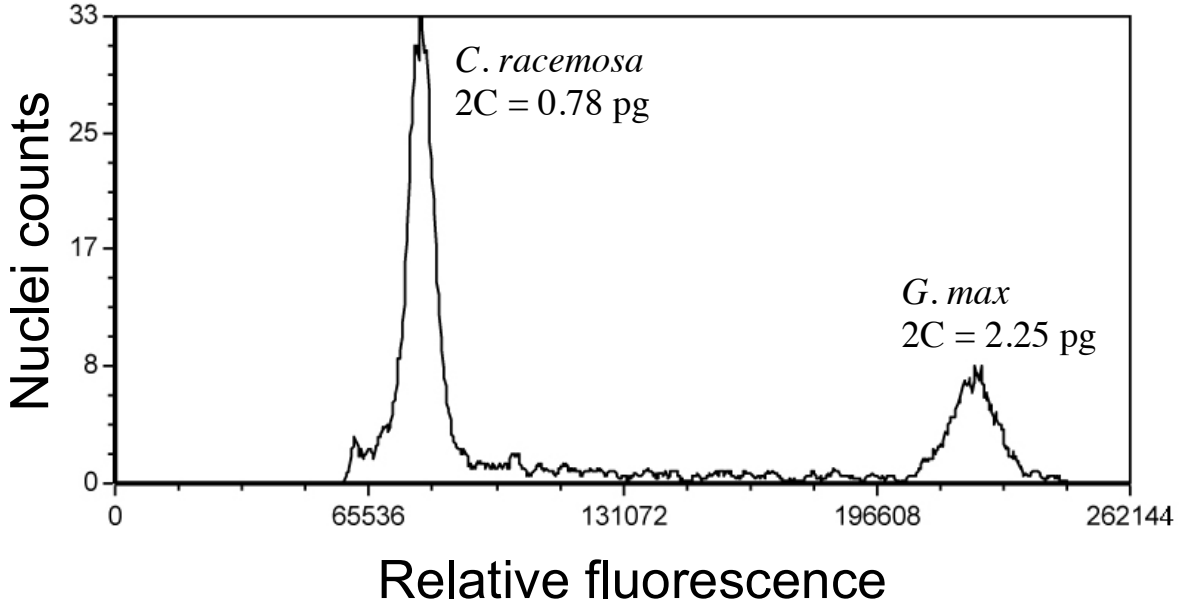

Figure 1. Histogram displaying mean fluorescence of stained nuclei, obtained from floral buds of *Cercis racemosa* Olive and leaf tissue of *Glycine max* L.

Chapter 2

Inheritance and Allelism of Morphological Traits in Eastern Redbud (*Cercis canadensis)*

(In the format appropriate for submission to *Horticulture Research*)

Inheritance and allelism of morphological traits in Eastern redbud (*Cercis canadensis*)

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Received for publication 16 September, 2015. Accepted for publication 26 September, 2015.

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Subject Category: Breeding, Cultivars, Rootstocks and Germplasm Resources.

Inheritance and allelism of morphological traits in Eastern redbud (*Cercis canadensis)*

Additional index words: Genetics, inheritance, allelism, plant breeding, tree architecture, transposable elements.

Abstract

Inheritance of purple, gold, and variegated foliage types, weeping architecture, and double flower was explored in F_1, F_2, and backcross families resulting from controlled hybridization of eastern redbud (*Cercis canadensis* L.). Potential allelic relationships were explored when possible. Inheritance analysis in families derived from controlled hybridization of 'Covey' (green leaf) and 'Forest Pansy' (purple leaf) suggest that purple leaf color and weeping architecture are both controlled by single recessive genes, for which the symbols *pl1* and *wp1* are proposed, respectively. Inheritance of gold leaf was explored in families of 'Covey' (green leaf) x 'Hearts of Gold' (gold leaf). Interpretation of inheritance of gold leaf in these families was confounded by the recovery of a leaf color phenotype in the F_2 family unlike either parent. However data suggested the action of a single locus controlling gold leaf color in 'Hearts of Gold', and that instability of gold leaf expression may be based on transposable element activity. Segregation of gold leaf in the F_2 families of 'Texas White' [green leaf (*C. canadensis* var. *texensis*)] x 'JN2' [gold leaf (The Rising Sun®)]did not fit a Mendelian ratio. Analysis of progeny of 'Silver Cloud' and 'Floating Clouds' (both showing white/green leaf variegation) with non-variegated cultivars

demonstrated that variegation in 'Silver Cloud' is controlled by a single recessive nuclear gene, while variegation in 'Floating Clouds' is controlled by cytoplasmic factors. The symbol *var1* is proposed for the gene controlling variegation in 'Silver Cloud'. Double flower in progeny derived from 'Flame' (double flower) suggested that double flower is dominant to single flower, and that 'Flame' is heterozygous at the double-flower locus, for which the symbol *Df1* is proposed. Allelism studies showed that the gene controlling purple leaf in 'Forest Pansy' is allelic to the purple leaf gene in 'Greswan' and that the gene controlling weeping phenotype in 'Traveller' (*C. canadensis* var. *texensis*) is non-allelic to the weeping gene found in 'Covey'. Allelism of the gold leaf trait in 'Hearts of Gold' and 'JN2' was investigated, but no clear conclusions regarding allelism could be made due to recovery of leaf color phenotypes unlike either parent.

Introduction

Eastern redbud (*Cercis canadensis* L.) is a small landscape tree that exhibits considerable morphological diversity, including variation in plant architecture, plant size, and flower and leaf colors. Specific cultivars and botanical varieties found in eastern redbud possess a variety of phenotypic characteristics whose inheritance can be studied and documented through strictly controlled breeding studies.[1] Determination of inheritance for these traits can help plant breeders better understand the genetic mechanisms that lead to specific phenotypes and allow greater control while manipulating these characteristics in a breeding program. Despite the relatively high number of observable characteristics, no named genes yet exist for any phenotypic variants found in eastern redbud. Furthermore,

little is known about modes of inheritance for these desirable traits, or allelic relationships between similar phenotypic variants that have arisen independently in different lineages.

The objectives of this study were to investigate the modes inheritance for purple, gold, and variegated (green and white) leaf color, weeping architecture, and double flower in *C. canadensis*. We further sought to determine if some of the aforementioned phenotypes found in different cultivars are caused by mutations at the same locus (allelic) or at different loci (non-allelic). Tests for allelism are valuable tools that allow plant breeders to determine if the genes responsible for certain traits are found at the same genetic locus of two or more accessions possessing similar phenotypes. By identifying particular genes as allelic, breeders can better predict how desirable traits will be expressed in future hybridizations.

There are several potential allelic relationships in *C. canadensis*. Two purple leaf cultivars of independent origin currently exist: 'Forest Pansy' and 'Greswan' (Burgundy Hearts®). 'Forest Pansy' was discovered in 1947 in Tennessee and 'Burgundy Hearts' was discovered in the early 2000s in Oklahoma, and both were chance seedlings. Two cultivars of independent origin that show gold leaf color are 'JN2' (The Rising Sun®) and 'Hearts of Gold'. Both cultivars have similar, but not identical phenotypes. 'Hearts of Gold' shows a solid gold leaf, with only slight anthocyanin expression in newly emerging leaves, whereas 'JN2' produces a gold leaf with orange overtones in the petiole and newly emerged leaves. As the leaves of 'JN2' mature they show numerous small, dark green spots scattered over the golden adaxial leaf surface (Figure 1).

Two weeping cultivars of independent origin were tested for allelism: Covey (Lavender Twist®), discovered in New York state in 1991, and Traveller (*C. canadensis* var.

texensis), discovered in Texas in 1989. The weeping phenotypes vary slightly between cultivars. 'Covey' demonstrates an abrupt weeping habit, whereas the weeping habit of 'Traveller' is slightly more open and spreading.

In this study, we describe the inheritance and allelism of various phenotypic traits using segregation data obtained from F_1, F_2, BC_{1P1}, and BC_{1P2} families. Furthermore, linkage among these traits was investigated when possible. Results presented in this manuscript were accumulated over 17 years of breeding efforts aimed at developing improved cultivars of redbud for the landscape and nursery industry.

Materials and Methods

Unless otherwise stated, all controlled hybridizations were performed under greenhouse conditions using potted trees and utilized identical pollination techniques. Because *C. canadensis* is self-incompatible, flowers did not require emasculation. Flowers on the female parent were pollinated using a fine artist's brush, using pollen obtained from shoots of the male parent forced in the greenhouse. Flowers were pollinated on the female parent when keel petals were fully extended, and separated from the banner and wing petals. Unless otherwise stated, all F_2 families were obtained by growing F_1 family trees in isolation at the Sandhills Research Station, Jackson Springs, North Carolina (USA). Isolation blocks were separated by at least 300 meters to minimize the potential for cross contamination with other families. Pollination among F_1 trees was accomplished by the presence of wild pollinating insects. Crosses, families generated, and progeny numbers are shown in Table 1.

Inheritance of purple leaf color and weeping architecture. Inheritance of purple leaf color and weeping architecture, and potential linkage between these traits, was investigated in F_1, F_2, BC_{1P1}, and BC_{1P2} families derived from the controlled hybridization of 'Covey' (green leaf, weeping architecture) and 'Forest Pansy' (purple leaf, non-weeping architecture). 'Covey' has a leaf color that is typical of wild type *Cercis* [(The Royal Horticultural Society Colour Chart, London, England), RHS green group 137A], and 'Forest Pansy' has leaf color RHS purple group N77A. The F_1 family was created by isolating a potted tree of both 'Covey' and 'Forest Pansy' within a pollination cage. A nest of bumblebees (*Bombus pennsylvanicus* De Geer) was placed inside the cage to accomplish cross pollination. The BC_{1P1} and BC_{1P2} families were created by utilizing controlled hybridization techniques previously described. The F_2 family was created by growing the 23 trees of the F_1 family in isolation as described above. F_2 seed was harvested from individual F_1 trees and kept separate, and F_2 progeny were later established in the field. Characterization of leaf color was conducted in early summer at a time when purple leaf color is highly expressed. Architecture (weeping vs. non-weeping) was scored on trees in their second growing season to ensure unambiguous characterization of weeping phenotypes. Preliminary studies in our program suggested that weeping growth habit and purple leaf color were each controlled by single recessive genes. Chi square analysis was used to test for goodness of fit to an expected ratio of 3:1 for leaf color (green leaf : purple leaf) and architecture (non-weeping : weeping), and to test for goodness of fit to an expected 9:3:3:1 ratio for a dihybrid cross involving both traits. Linkage between the purple leaf and weeping architecture was tested using contingency analysis. Since four different F_2 families were utilized in this study (each

derived from separate full-sib F_1 trees), data from each family were tested for departure from homogeneity.

Inheritance of gold leaf color- 'Hearts of Gold'. Inheritance of the gold leaf trait was explored in F_1, F_2, BC_{1P1}, and BC_{1P2} families derived from hybridization of 'Covey' (green leaf) × 'Hearts of Gold' (gold leaf). Families were generated as previously described. Progeny were scored for leaf color in the greenhouse shortly after germination, at the second true leaf stage. We initially hypothesized that F_2 progeny would segregate into only green leaf and gold leaf categories. However some individuals showed a leaf color phenotype unlike either parent, classified as "bleached", showing cotyledons and first true leaves that were white to light yellowish-green with sparse green streaking. Hence, segregating progeny were separated into one of three categories based on leaf color (green, gold, and bleached). Since five different F_2 families were utilized in this study (each derived from separate full-sib F_1 trees), data from each family were tested for departure from homogeneity.

Inheritance of gold leaf color - 'JN2' (The Rising Sun®). Inheritance of the gold leaf phenotype was further explored in F_1, F_2, BC_{1P1}, and BC_{1P2} families derived from hybridization of 'Texas White' (*C. canadensis* var. *texensis*, green leaf) and 'JN2' (gold leaf with small green spots). In all families, ratios were calculated for the segregating families and tested using the Chi-square test for goodness of fit for leaf color. For F_2 analysis, two approaches were undertaken. In the first case, F_2 seed was harvested from approximately 100 randomly chosen F_1 trees and bulked (bulked F_2 family). In the second approach, about

50-75 seed were harvested from 38 randomly selected F_1 trees, and kept separate for analysis. Progeny from 5 of these 38 separate F_2 families failed to segregate for both gold leaf color and gold/green (mottled) leaf color. Thirteen of the F_2 families produced only green and mottled leaf progeny, lacking gold leaf segregants, prompting the authors to investigate whether or not the F_1 parents of the families not segregating for gold leaf were true hybrids of 'Texas White' and 'JN2'. Simple sequence repeat (SSR) loci were utilized to determine the genetic identity of 12 of the F_1 parents not segregating for gold leaf color. Leaf tissue was collected from both parental genotypes ('JN2' and 'Texas White') and 12 selected intra-specific hybrids and stored at -80 °C until genomic DNA isolation. Tissue was homogenized by grinding in liquid nitrogen and DNA isolated using the Qiagen Dneasy Plant DNA isolation kit (Qiagen, Valencia, California, USA). The manufacturer's instructions were followed for DNA isolation except that 1.5 % PVP was added to Buffer AP1. Total DNA was quantified with the NanoDrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA), DNA quality was determined using 2% agarose gels stained with ethidium bromide and visualized in the 2000 Gel Documentation System (Bio-Rad Laboratories, Hercules, California, USA).

Primer pairs from *C. canadensis* microsatellite loci[2] that were polymorphic between 'JN2' and 'Texas White' were selected and screened against 12 putative hybrids to confirm true hybrid origin. Microsatellite amplification was completed using the following conditions: 10 µL PCR reactions contained 0.4 ng genomic DNA, 2.5 mM $MgCl_2$, 1× GeneAmp PCR Buffer II (Applied Biosystems, Foster City, CA), 0.2 mM dNTPs, 0.25 µM primer, 0.6 U AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City,

California, USA), and sterile, nanopure water. Cycling conditions were as follows: 1 cycle of 94 ºC for 5 min; 35 cycles of 94 ºC for 40 s, 55 ºC for 40 s, 72 ºC for 30 s, and 1 cycle of 72 ºC for 4 min. PCR products were sized on the QIAxcel Capillary Electrophoresis System (Qiagen, Valencia, California, USA) using an internal 25-bp DNA step ladder.

Raw allele length data for each sample and locus were binned into allelic classes using the program FLEXIBIN[3]. We utilized a conservative 2 bp allelic class size range because of the 2 bp resolution of the QIAxcel Capillary Electrophoresis System. All loci selected for hybrid confirmation were polymorphic and differed by at least 4 bp in the parents to eliminate potential resolution issue during allele separation. The multilocus genotypic data for each hybrid was compared to the parents for hybrid confirmation. To simplify hybrid confirmation, only loci that were homozygous in each respective parent were utilized. Thus, every hybrid should have two alleles detected for each locus analyzed.

Inheritance of double flower. Inheritance of the double flower phenotype (Figure 2) was investigated in F_1 progeny derived from controlled hybridization of 'Dwarf Alba' (single flower) × 'Flame' (double flower). The double-flowered 'Flame' is essentially female sterile, and rarely sets fruit. The double-flowered F_1 progeny recovered from the aforementioned hybridization were also female sterile, precluding the recovery of F_2 progeny. Compared to the typical 5 petals in a flower of redbud, 'Flame' typically shows 30-40 petals per flower (unpublished data). Atypically, in 2005 a tree of 'Flame' growing among a diverse collection of redbud cultivars set fruit. Hence, viable seed was collected and obtained from Hidden

Hollow Nursery (Belvidere, Tennessee, USA) and open pollinated seedlings were evaluated to obtain additional evidence on inheritance of double flower.

Inheritance of variegated leaf. Inheritance of the variegated leaf phenotype was investigated in five different F_1 families generated from the hybridization of 'Floating Clouds' (variegated leaf) with non-variegated cultivars 'Covey' (cross made reciprocally), NC2006-14, 'Texas White' and 'JN2'. Inheritance of variegated leaf was investigated further in F_1 and F_2 families derived from reciprocal hybridizations of 'Silver Cloud' (variegated leaf) and 'Covey'. In both cases, ratios were calculated for the segregating families and tested using the Chi-square test for goodness of fit. Both 'Floating Clouds' and 'Silver Cloud' have variegated green leaves with white sectors. However, both were discovered as chance seedlings of independent origin. Their phenotypes are similar, but 'Floating Clouds' has more prominent white sectors and leaves are more resistant to sunscald (personal observation).

Allelism of purple leaf phenotypes. Preliminary analysis suggested that purple leaf color in 'Forest Pansy' is controlled by a single recessive gene. In order to determine if the genes controlling purple leaf color in 'Forest Pansy' and 'Greswan' are allelic, controlled hybridizations were accomplished under greenhouse conditions. Because both 'Forest Pansy' and 'Greswan' show a high degree of female sterility, 'Greswan' was utilized as the male parent, and the purple leaf cultivar 'Ruby Falls' (female fertile), derived from 'Forest Pansy' was used as the female parent. If controlled by the same locus, and if both cultivars

are homozygous for the recessive mutation, one would predict all F_1 progeny to exhibit purple leaf color. Conversely, if the purple phenotype is controlled by different loci in the two cultivars, one would expect all F_1 progeny to exhibit green leaf color. Progeny were scored for purple leaf color in the greenhouse, after development of two true leaves. Additionally, open pollinated seed of 'Greswan' were obtained from trees growing at the JC Raulston Arboretum (Raleigh, North Carolina, USA) and from Green Leaf Nursery, (Park Hill, Oklahoma, USA) to verify if the purple leaf phenotype exhibited by 'Greswan' is recessive. Because eastern redbud is self-incompatible, all open-pollinated seed originate from outcrossing. Open-pollinated seedlings should show green leaves if the purple leaf phenotype is recessive in 'Greswan', assuming no outcrossing to purple leaf plants.

Allelism of weeping phenotypes. Allelism of the genes controlling weeping phenotype in 'Covey' and 'Traveller' was investigated. Because 'Traveller' shows almost absolute male and female sterility, it cannot be used in controlled crosses. However, a rare open-pollinated seedling of 'Traveller' (designated NC2011-1) was obtained in our program in 2011. The seedling is non-weeping, but presumably heterozygous for the weeping allele. Hence, hybridization between NC2011-1 and 'Ruby Falls' (derived from 'Covey') is informative for allelism determination, and reciprocal crosses were made in the greenhouse. If the weeping phenotypes are controlled by allelic genes, one would predict recovery of 50% weeping progeny in the F_1 family. Alternatively, if the weeping genes are non-allelic, one would expect only non-weeping offspring in the F_1 family. Initial scoring of progeny was performed approximately seven months after germination, at the end of the first growing

season. Subsequently, all progeny were again scored in mid-summer of the second growing season to confirm accuracy of scoring.

Allelism of gold leaf phenotypes. Both gold-leaf cultivars (Hearts of Gold and JN2) were crossed in reciprocal combinations to investigate allelism of the genes controlling gold leaf color. Controlled hybridizations were made in a greenhouse, and progeny were scored for leaf color in the first growing season, in both a greenhouse and field setting. If controlled by the same locus, and if both cultivars are homozygous for the recessive mutation, one would predict all F_1 progeny to exhibit gold leaf color. Conversely, if the gold leaf phenotype is controlled by different loci in the two cultivars, one would expect all F_1 progeny to exhibit green leaf color.

Results and Discussion

Inheritance of purple leaf color and weeping architecture. The F_1 family of 'Covey' × 'Forest Pansy' consisted of 23 plants, all showing non-weeping growth habit and green leaves. The F_2 seed was derived from random intercrossing of 23 F_1 trees and collected from 4 separate F_1 trees, providing 1,586 progeny. Leaf color of F_2 progeny could be easily discerned in early stages of development, typically by observing the first true leaves. Cotyledons of purple leaf plants were green, identical to the cotyledons of green leaf progeny. Segregating F_2 progeny exhibiting the purple leaf phenotype were clearly discernable from those exhibiting the green phenotype in early summer, the time of scoring in the field (Figure 3). Characterization of the weeping phenotype in F_2 progeny proved to

be more difficult than the purple leaf phenotype in early stages of development. Scoring progeny in the second growing season was considerably easier than doing so in year one. Numerous progeny, particularly in the first growing season, exhibited an intermediate phenotype between weeping and non-weeping. Assuming single gene action, these progeny possibly represent heterozygotes for the weeping gene. As a result, only those individuals that clearly demonstrated the rigid and distinct weeping phenotype as shown by 'Covey' (Figure 4) were categorized as weeping.

A Chi-square test for heterogeneity among the four F_2 families for segregation of both purple leaf color and weeping architecture was non-significant (P=0.09 and P=0.87, respectively), so data was combined prior to analysis. Segregation for leaf color fit the expected ratio of 3:1 (green leaf : purple leaf) at P=0.02 (Table 2). A slight underrepresentation of purple leaf progeny accounted for the minor distortion in the F_2 data. The F_1 and F_2 data indicate that purple leaf is recessive to green leaf, and that purple leaf is controlled by a single recessive gene. This conclusion is supported by backcross data (Table 2), showing a lack of purple leaf segregants in the BC_{1P1} family. BC_{1P2} progeny derived from the backcross of ('Covey' × 'Forest Pansy') × 'Forest Pansy' segregated in the expected ratio of 1:1 (green leaf : purple leaf) at P=0.82 (Table 2). We propose that purple leaf color in 'Forest Pansy' is controlled by a single recessive gene, designated *pl1*. Furthermore, we also propose that 'Forest Pansy' has a genotype *pl1pl1* and that those plants exhibiting the wild-type green leaf have a genotype *Pl1Pl1*.

Analysis of combined data for the four F_2 families showed segregation for the weeping phenotype fit a ratio of 3:1 (non-weeping : weeping) at P=0.26 (Table 3). Although

all F_1 plants were classified as non-weeping, they did demonstrate a slight semi-pendulous growth habit in their first two years of growth, subsequently less distinct as trees aged. The F_1 and F_2 data indicate that weeping architecture is recessive to non-weeping, and weeping is controlled by a single recessive gene for which we propose the designation *wp1*. We propose that 'Covey' has a genotype of *wp1wp1*. Our hypothesis that weeping is recessive to non-weeping was further supported through BC_{1P1} segregants which fit the expected ratio of 1:1 (non-weeping : weeping) at P=0.44 (Table 3) and BC_{1P2} segregants which showed a lack of weeping progeny. Results of contingency analysis suggest that the genes responsible for purple leaf color and weeping architecture are not linked (P=0.69, Table 4). Additionally, combined co-segregation analysis for the purple leaf and weeping traits fit a ratio of 9:3:3:1 (P=0.05, Table 5) that would be predicted for a di-hybrid cross involving two recessive genes. This further supports lack of linkage between the purple leaf and weeping loci.

Inheritance of gold leaf color - 'Hearts of Gold'. The F_1 family derived from 'Covey' × 'Hearts of Gold' consisted of 37 plants, all non-weeping with green leaves, expected if the gold leaf trait is simply inherited and recessive. The 37 F_1 trees were grown in isolation and were randomly intercrossed by natural pollinators, and F_2 seed was harvested from five F_1 trees and kept separate. We initially predicted that F_2 progeny would segregate into only the parental green leaf and gold leaf categories. However some segregants demonstrated a leaf color phenotype unlike either parent, classified as bleached. Bleached segregants produced small, distorted leaves, nearly albino, but with sparse light yellow spots (Figure 5). Typically, bleached progeny did not survive past the seedling stage. The F_2 and backcross plants were

separated into one of three categories based on leaf phenotype (green, gold, and bleached), which was assessed at the second true leaf stage. Cotyledon color was highly predictive of leaf color. Green leaf plants expressed a cotyledon color typical of wild type (RHS green group 137A), (Figure 6). Plants scored as gold leaf had cotyledons that were distinguishably lighter in color (RHS yellow-green group 144A) than the green group. The bleached category possessed cotyledons that were very pale yellow (RHS greyed-yellow group 160 D). Those bleached progeny that survived in the greenhouse had very light yellow leaves that became necrotic upon exposure to full sun.

The segregation in the F_2 of green, gold, and bleached progeny did not match any known Mendelian segregation ratio, perhaps suggesting the interaction of more than one locus. However, when all non-green segregants were combined into a single category (gold + bleached), each of the five F_2 families fit a ratio of 3:1 (green leaf : gold + bleached leaf) (Table 6). A chi square test for heterogeneity among the five F_2 families was conducted, using combined data for gold and bleached as a single phenotypic category. The heterogeneity chi-square was non-significant (P=0.55), so data were combined prior to analysis. Combined data fit a ratio of 3:1 (green leaf : gold + bleached leaf) at P= 0.06 (Table 6). Demonstration of a 3:1 ratio (green leaf : gold + bleached leaf) after combination of gold and bleached leaf phenotypes is suggestive of the action of only one locus controlling both the gold leaf and bleached phenotypes. Although this segregation ratio is more consistent with inheritance involving a single gene recessive trait, it does not explain the recovery of numerous bleached progeny. One possible explanation is transposable element (TE) activity at the locus controlling gold leaf, leading to a small subset of progeny

producing a very low amount of chlorophyll (bleached phenotype). Insertion of non-autonomous DNA based active rice transposon one (nDart1) has resulted in pale yellow variegation in rice, and mutants of *Arabidopsis thaliana* that underwent *Ds* insertion events have resulted in albino phenotypes.[4,5] Similarly, *DS* insertion mutants of *Arabidopsis* experienced a disruption of ribosome release factor 1, which proved to be critical in chloroplast development and PSII activity.[6] The observed ratio of 3:1 (green leaf : gold + bleached leaf) coupled with the presence of limited chlorophyll in the cotyledons of bleached progeny suggests that the bleached phenotype may be gold leaf segregants that experienced a TE insertion or excision event early in development, rendering them highly disabled in chlorophyll synthesis. Several instances in our study were documented in which a viable bleached segregant ultimately developed into a very light gold phenotype, reflecting the transient nature of TE based phenotypes. TE activity could be the cause for the moderate degree of phenotypic instability witnessed in some F_2 progeny derived from 'Covey' × 'Hearts of Gold' (Figure 7).

The BC_{1P1} family showed only green leaf progeny, as predicted if gold leaf is recessive. Segregants from the BC_{1P2} family fell into the same three phenotypic categories found in the F_2 (green, gold and bleached). Even when gold and bleached categories were combined into a single group (gold + bleached), this family showed a deficiency of non-green leaf progeny that resulted in a distortion of the expected 1:1 ratio (green leaf : gold + bleached leaf), at $P<0.001$ (Table 6). The basis for this distortion cannot be explained. These results confirm that the gold leaf trait in 'Hearts of Gold' is not cytoplasmic, as 'Hearts of Gold' was used as the male parent in the initial controlled hybridization that created the F_1

family. Additionally, our data shows that progeny derived from ‘Hearts of Gold’ exhibited a moderate degree of phenotypic instability, more so than progeny derived from the other gold leaf cultivar, JN2.

Inheritance of gold leaf color - 'JN2' (The Rising Sun®). The F_1 family of ‘Texas White’ (green leaf) × ‘JN2’ (gold leaf with green spots) consisted of 463 progeny, all of which showed green leaf color. The F_1 progeny were intermated (natural pollinators), and F_2 seed was collected from about 100 F_1 trees and bulked. The bulk F_2 family segregated for both green, entire gold (no green spots) and mottled leaf phenotypes (Figure 8). The mottled leaf phenotype was similar to gold leaf, but showed an overlay of small, mottled green sectors, similar to the phenotype typically expressed on leaves of ‘JN2’, the gold leaf parent. Considerable variation existed among the mottled phenotype plants, ranging from subtle to pronounced. No bleached progeny were recovered among F_2 segregants in the 'Texas White' × 'JN2' family, unlike the 'Covey' × 'Hearts of Gold' F_2 family, in which bleached progeny were recovered in moderate numbers. Cotyledon color was highly predictive of leaf color in segregating families. Green leaf plants expressed a cotyledon color typical of wild type (RHS green group 137A). Plants scored as gold leaf had cotyledons that were distinguishably lighter in color (RHS yellow-green group 144A). The mottled category possessed cotyledons that were identical to the gold cotyledon classification. The F_2 and backcross plants were separated into one of three categories based on leaf phenotype (green, gold, and mottled), which was assessed at the second true leaf stage. Segregation in the F_2 of green, gold, and mottled progeny did not match any known Mendelian segregation ratio,

perhaps suggesting the interaction of more than one locus. As with the 'Covey' × 'Hearts of Gold' study, all non-green segregants were pooled into a single category (gold + mottled) and analyzed for a goodness of fit to a 3:1 ratio (green leaf : gold + mottled leaf). However, analysis of the bulk F_2 family did not fit the expected ratio of 3:1 (green leaf : gold + mottled leaf) at $P<0.001$, showing a highly significant underrepresentation of the gold+ mottled progeny category (Table 7).

Subsequently, in order to better characterize the segregation distortion in the bulk F_2 family, a second experiment was conducted. For this experiment, additional F_2 seed was collected from 38 individual F_1 trees, and kept separate for analysis. An average of 55 F_2 progeny were grown on from each of these individual F_1 trees to further assess leaf color segregation. Consistent with the results of the bulked F_2 family, green, gold, and mottled progeny were recovered. There was a significant deviation ($P=0.05$) in 24 of the 38 families from the expected 3:1 ratio (green leaf : gold + mottled leaf), again with a deficiency in "gold + mottled" progeny (Table 8). Gold leaf F_2 segregants in these families exhibited uniform gold leaf color, more similar to 'Hearts of Gold' and distinct from the mottled gold phenotype of 'JN2'. Unexpectedly, five of the 38 F_2 families (10, 28, 31, 33, 38) failed to show any gold leaf or mottled segregants (303 total segregating progeny). Based on a single gene model (with gold leaf being recessive to green leaf), the likelihood of recovering only green leaf segregants from a population of 303 segregating progeny is 1.39×10^{-38}. Thirteen additional F_2 families (8, 9, 13, 14, 15, 29, 30, 32, 34, 36, 39, 49, 50) showed no gold progeny, but only green and mottled progeny.

All progeny derived from the BC_{1P1} family ['Texas White' × ('Texas White' × 'JN2')] were green leaf (Table 7), supporting the hypothesis that gold leaf is recessive to green leaf. The BC_{1P2} family ['JN2' × ('Texas White' × 'JN2')] segregated for both green and gold leaf, but the mottled phenotype was not recovered. Segregation in the BC_{1P2} family deviated significantly from the expected test ratio of 1:1 (green leaf : gold leaf) (P=0.007), showing a deficiency of gold leaf segregants (Table 7). Results from backcross families are suggestive of a single recessive gene controlling gold leaf in 'JN2', but the highly significant underrepresentation of gold leaf and mottled progeny recovered in the F_2 and BC_{1P2} families is difficult to explain. In this particular BC_{1P2}, the heterozygous F_1 was used as the male parent. It is possible that pollen carrying the gold leaf allele does not effectively compete with pollen carrying the green leaf allele, resulting in a deficiency of gold leaf segregants. A similar phenomenon has been observed in *Pisum sativum*, where pollen carrying the stringless allele grows more slowly than wild-type pollen, resulting in a deficiency of stringless progeny in F_2 and certain backcross families.[7] However, this explanation does not adequately explain the total absence of gold leaf and mottled segregants in five of the sampled F_2 families.

Alternatively, the distortion in segregation ratios in the bulked F_2 family, and the lack of gold leaf segregants in five of the 38 individual F_2 families could be explained if contamination occurred during pollination to create the F_1 family, resulting in non-hybrid progeny being sampled. To rule out this possibility, the five F_1 trees that failed to segregate for gold leaf or mottled progeny were analyzed along with seven additional F_1 trees that segregated only for green leaf and mottled progeny. Six microsatellite loci (386b, 508a,

658a, 671a, 732a, 830a) were used to confirm hybridization between the parents ‘JN2’ and ‘Texas White’. These loci were homozygous and polymorphic between the parents. All of the putative hybrids (NCTWRS-9, NCTWRS-10, NCTWRS-14, NCTWRS-28, NCTWRS-31, NCTWRS-32, NCTWRS-33, NCTWRS-34, NCTWRS-36, NCTWRS-38, NCTWRS-39, and NCTWRS-50) tested were heterozygous and had a single allele from each parent, thus confirming hybridization between ‘Texas White' and ‘JN2'

Since all tested F_1 trees proved to be true hybrids, one would predict them to be heterozygous for the gold leaf allele derived from 'JN2'. That five of these families failed to segregate for gold or mottled leaf color, and 13 additional families (seven verified as true hybrids using SSR's) segregated only for mottled but not gold progeny suggests that some factor is impacting transmission of the gold leaf allele. Activity of TE could be responsible for the distortion in the observed ratios and complete lack of gold leaf and mottled progeny in specific F_2 families. If the gold leaf mutation is based on a TE insertion, a TE excision event during male gametogenesis could restore function and result in a modified allele that functionally behaves as a green leaf allele, resulting in F_1 progeny that are homozygous for green leaf. These homozygous green F_1 progeny could account for the underrepresentation of gold leaf and mottled segregants in the bulk F_2 family, and lack of gold and mottled segregation in F_2 families derived from individual F_1's.

The leaf phenotype of the 'JN2' parent, showing a predominantly gold leaf with numerous random islands of green of varying size, is suggestive of TE activity. The mottled leaf phenotype recovered in progeny of ‘JN2’ could be the result of a partial TE excision at the gold allele during gametogenesis or early embryo development, resulting in partial

restoration of function and creation of a unique leaf color phenotype similar to 'JN2', and unlike the green leaf 'Texas White' parent. Recovery of solid gold progeny lacking green spots in F_2 family may also represent TE element based variation. A similar phenomenon may explain the bleached phenotype in the 'Covey' × 'Hearts of Gold' F_2 progeny.

Assuming maternal inheritance of chloroplasts and mitochondria in redbud, our results confirm that the gold leaf trait in 'JN2' is not inherited cytoplasmically as 'JN2' was used as the male parent in the initial controlled hybridization that created the F_1 family.

Inheritance of double flower. Two F_1 progeny derived from the hybridization of 'Dwarf Alba' × 'Flame' were both double flowered, suggesting dominance of double flower. Analysis of F_1 progeny derived from open pollination of 'Flame' showed segregation for single flower and double flower. Segregation for single and double flower did not fit the expected test ratio of 1:1 (single flower : double flower) at $P<0.001$ (Table 9), showing a deficiency of single flowered progeny. However, the data suggest that double flower is controlled by a single dominant gene, for which we propose the designation *Df1,* and that 'Flame' is heterozygous at this locus.

Inheritance of variegated leaf - 'Floating Clouds' and 'Silver Cloud'. Five F_1 families generated from hybridizations involving 'Floating Clouds' (variegated leaf) showed that leaf variegation in that cultivar is inherited cytoplasmically (Table 10). In the four instances where 'Floating Clouds' was utilized as the female parent, all F_1 progeny exhibited variegated foliage In the one instance in which 'Floating Clouds' was the male parent in a

hybridization with 'Covey', all F_1 progeny exhibited green foliage.

Analysis of the F_1 and F_2 families derived from the hybridization of ‘Covey’ × ‘Silver Cloud’ show that variegation in 'Silver Cloud' is controlled by a nuclear gene, with the two F_1 progeny both showing green leaves. These F_1 progeny were intermated, and seed was harvested off of each tree separately yielding two F_2 families. Segregation analysis of the F_2 families (NC99-18-1 and NC99-18-2) fit the expected test ratio of 3:1 (green leaf : variegated leaf) at P=0.02 and P=0.74, respectively (Table 11). These findings indicate that variegation from 'Silver Cloud' is controlled by a single recessive gene for which we propose the designation *var1*. Hence, the genotype of 'Silver Cloud' is *var1var1*.

Allelism of purple leaf phentoype. F_1 progeny derived from hybridization of ‘Ruby Falls’ × ‘Greswan’ all showed purple leaf color, except for a single individual, which was green leaf. Progeny derived from open pollination of 'Greswan' in a field setting were green leaf, with the exception of three purple leaf individuals (Table 12). Because eastern redbud is self-incompatible, all open-pollinated seedlings derived from open pollination of 'Greswan' are half-sibs, confirming that the purple leaf trait in 'Greswan' is recessive. Hence, a preponderance of purple offspring derived from the allelism test hybridization are not caused by dominance of purple leaf color in 'Greswan'. The presence of three purple leaf progeny in the open-pollinated family derived from ‘Greswan’ could be explained by outcrossing to another purple leaf cultivar. These results strongly support that the genes controlling purple leaf color in 'Forest Pansy' and 'Greswan' are allelic. We propose that genes conferring

purple leaf color in ‘Greswan’ be designated *pl1pl1* in accordance with the previous designation established for ‘Forest Pansy’.

Allelism of weeping architecture. Progeny derived from reciprocal hybridizations between NC2011-1 (non-weeping and heterozygous for the weeping gene derived from 'Traveller') and ‘Ruby Falls’ (homozygous for the weeping gene derived from 'Covey') were all non-weeping except for a single weeping individual, recovered in the family derived from NC2011-1 as the female parent. This individual is presumably the result of a rare self-pollination event in NC2011-1. A total of 316 progeny were evaluated in the reciprocal families combined. Recovery of 50% weeping progeny would have been expected if the genes were allelic, hence these results show the genes are non-allelic. Alternatively, it is possible that the gene that confers weeping in ‘Traveller’ is dominant and that ‘Traveller’ is heterozygous at that locus. If this were the case, NC2011-1 may possess the recessive (non-weeping) form of the allele, which would render all F_1 progeny derived from hybridization with ‘Ruby Falls’ non-weeping as well. However, the recovery of a weeping individual from the cross using NC2011-1 as the female parent argues against this possibility. Because the weeping phenotypes in these two cultivars differ slightly, the potential exists that unique weeping forms will be recovered in the F_2 generation, based on selection of progeny expressing weeping genes from both parents.

Allelism of gold leaf phenotype. Reciprocal crosses were made between gold leaf cultivars Hearts of Gold (entire gold) and JN2 (gold with green spots) to test for allelism of

the gold leaf trait. We expected to recover either all green leaf (genes non-allelic) or all gold leaf (genes allelic) progeny from these crosses, but both green and gold leaf progeny were recovered, contradictory to a simple affirmation or rejection of allelism. In addition, two other leaf color phenotypes were observed. The bleached phenotype, described earlier in the manuscript, was recovered, as well as a fourth category, designated as chartreuse. Plants classified as chartreuse exhibited a cotyledon color that was intermediate between the gold and bleached categories. Chartreuse plants showed very light gold color in mature leaves (RHS greyed-yellow group 160A). Interestingly, cotyledon color varied considerably among segregating offspring, and appeared to be highly associated with leaf color, hence progeny were initially placed into the four phenotypic categories (green, gold, bleached, and chartreuse) based on cotyledon color. Plants were then grown on for 2-4 months and scored again based on leaf color (Figure 9), so that the accuracy of scoring leaf color phenotypes at the cotyledon stage could be assessed. In the case of both families, the most frequently occurring phenotypes fell into the gold and green leaf categories.

The inherent difficulty of accurately scoring progeny at the cotyledon growth stage is illustrated by the change in frequency of progeny in each category upon final scoring of leaf color (Table 13). The majority of 'Hearts of Gold' × 'JN2' F_1 progeny originally designated in the cotyledon stage as green maintained this phenotype, however, a small percentage were later determined to belong to either the gold or chartreuse phenotypes. A small percentage of the progeny scored initially as gold were later determined to belong to either the green or chartreuse categories.

The majority of progeny classified initially in the cotyledon stage as chartreuse developed into plants with very light gold colored leaves and an airbrushed overtone of very small, numerous, light green spots, similar to but distinct from the "mottled" phenotype of 'JN2'. Of the bleached progeny derived from ‘JN2’ × ‘Hearts of Gold’, 56% proved to be seedling-lethal. The remaining bleached progeny that survived past the seedling stage in the greenhouse ultimately developed into plants later reclassified as chartreuse. Upon placement in a field setting, all chartreuse segregants showed significant leaf burn and perished. These results suggest that plants classified as chartreuse may simply have a different degree of expression of the bleached phenotype. A moderate number of progeny in this study exhibited considerable phenotypic instability, demonstrating green and gold sectoring on cotyledons and/or leaves of individual plants (Figure 7). This instability was observed in all phenotypic categories.

Progeny that were generated through reciprocal crosses of these two gold leaf parents did not conform to ratios that would be expected from simple allelic or non-allelic inheritance patterns. Consistent with families generated for the gold leaf inheritance study derived from 'Hearts of Gold', reciprocal crosses of ‘Hearts of Gold’ and ‘JN2’ yielded not only green leaf and gold leaf progeny, but bleached and chartreuse progeny as well (Figure 9). One would have expected all gold leaf progeny if the genes were allelic, or conversely, all green leaf if the genes were non-allelic. The recovery of multiple leaf color phenotypes ranging from near albino, to light and bright gold, to green, and the observed phenotypic instability for leaf color in individual plants is highly suggestive of instability conferred by TE activity in the gold leaf locus. It is possible, given the relatively high number of gold leaf

segregants, that genes coding for gold leaf color in ‘Hearts of Gold’ and ‘JN2’ are indeed allelic, but transposon mediated events during gametogenesis or early embryo development impacted phenotypic expression, leading to the observed phenotypes and instability of expression. Both ‘Hearts of Gold’ and ‘JN2’ demonstrate classic signs of transposon activity. As previously mentioned, ‘JN2’ exhibits small islands of green scattered across a gold leaf surface, reminiscent of the "broken colors" variegation found in the flowers of *Mirabilis jalapa* L.*,* a species known for transposon mediated variegation.[8] The mutagenic nature of transposons can be suppressed by small interfering RNA’s, especially in gametes that could transmit transposed elements to the next generation. Also, both mobility and expression of TEs can be transiently activated in the vegetative nuclei (tube nuclei) of pollen, which can ultimately lead to new somatic transpositions.[9] The bleached, green and chartreuse phenotypes recovered from hybridizations involving ‘Hearts of Gold’ and ‘JN2’ could be the result of such transient activation. TE activity in pollen of ‘Hearts of Gold’ or ‘JN2’ could cause unpredictable transmission of the gold leaf phenotype in different genetic backgrounds. It is possible the bleached and chartreuse progeny recovered in these families were gold leaf segregants whose phenotypes were each altered by TE activity in a different way.

Conclusions

The modes of inheritance for several phenotypic mutations in *Cercis canadensis* have been documented. The purple leaf phenotype is controlled by a single, recessive gene (*pl1pl1*) in 'Forest Pansy' allelic to the purple leaf gene in ‘Greswan’. Weeping architecture in ‘Covey’ is controlled by a single recessive gene (*wp1wp1*), non-allelic to the mutation

conferring weeping architecture in ‘Traveller’. Inheritance of the variegated leaf trait in 'Silver Cloud' and 'Floating Clouds' revealed that variegation in 'Silver Cloud' is controlled by a single recessive nuclear gene (*var1var1*), while variegation in 'Floating Clouds' is controlled by cytoplasmic factors. The double flowered phenotype of 'Flame' is controlled by a single dominant gene (*Df1Df1*). Inheritance of the gold leaf phenotype found in ‘Hearts of Gold’ and ‘JN2’ is less well understood but likely the result of one major recessive gene. The gold leaf phenotype in both ‘Hearts of Gold’ and 'JN2' was transmitted both paternally and maternally. The genes controlling gold leaf in these two cultivars are likely allelic, but this could not be definitively determined. Phenotypic instability in leaf color observed in F_2 progeny derived from 'Hearts of Gold', and from F_1 progeny derived from 'Hearts of Gold' × 'JN2' is highly suggestive of TE activity, which could explain the segregation distortion and range of phenotypic variation in leaf color revealed in hybridizations involving 'Hearts of Gold' and 'JN2'. It is possible that the bleached, mottled, and chartreuse phenotypes recovered in families derived from these two cultivars represent variable expression of the gold leaf phenotype altered by TE activity and genetic background.

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Acknowledgments

The authors gratefully acknowledge the technical assistance of Mr. Layne Snelling, Mr. Chris Harlow, and the staff at the Sandhills Research Station, Jackson Springs, North Carolina. Research supported by funds provided by the North Carolina Agricultural Research Service (NCARS), North Carolina Nursery and Landscape Association (NCNLA), J. Frank Schmidt Family Charitable Foundation, PlantHaven International, and the U.S. Department of Agriculture (Grant # 58-6404-1637). Thanks to Hidden Hollow Nursery, Belvidere, Tennessee for assistance with propagation of experimental materials and providing open-pollinated seed of 'Flame' redbud. Thanks to Mark Andrews, Green Leaf Nursery, Park Hill, Oklahoma for providing open-pollinated seed of 'Greswan' (Burgundy Hearts®) redbud.

Table 1. *Cercis canadensis* crosses and progeny numbers screened for inheritance of purple leaf color, weeping architecture, gold leaf color, double flower and variegated leaf.

Cross	Inheritance of trait(s)	Families generated (no. of progeny)[Z]
'Covey' × 'Forest Pansy'	Purple leaf / weeping	F_1 (23), F_2(1586)
'Covey' × ('Covey' x 'Forest Pansy')	Purple leaf / weeping	BC_{1P1}(112)
('Covey' × 'Forest Pansy') × 'Forest Pansy'	Purple leaf / weeping	BC_{1P2}(318)
'Covey' × 'Hearts of Gold'	Gold leaf	F_1(37), F_2(1116)
'Covey' × ('Covey' × 'Hearts of Gold')	Gold leaf	BC_{1P1}(123)
'Hearts of Gold' × ('Covey' × 'Hearts of Gold')	Gold leaf	BC_{1P2}(349)
'JN2' × 'Texas White'	Gold leaf	F_1(8)
'Texas White' × 'JN2'	Gold leaf	F_1(463), F_2 bulk(1195), F_2(2,216)
'Texas White' × ('Texas White' × 'JN2')	Gold leaf	BC_{1P1}(877)
'JN2' × ('Texas White' × 'JN2')	Gold leaf	BC_{1P2}(108)
'Dwarf Alba' × 'Flame'	Double flower	F_1(2)
'Flame' (open pollinated)	Double flower	OP(260)

Table 1 Continued.

'Covey' × 'Floating Clouds'	Variegated leaf	F1(41)
'Floating Clouds' × 'Covey'	Variegated leaf	F1(5)
'Floating Clouds' × 'Texas White'	Variegated leaf	F1(127)
'Floating Clouds' × 'JN2'	Variegated leaf	F1(61)
'Floating Clouds' × NC2006-14	Variegated leaf	F1(15)
'Covey' × 'Silver Cloud'	Variegated leaf	F1(2), F2(1771)
'Ruby Falls' × 'Burgundy Hearts'	Purple leaf allelism	F1(196)
'Burgundy Hearts' (open pollinated)	Purple leaf allelism	OP(91)
NC2011-1 × 'Ruby Falls'	Weeping allelism	F1(117)
'Ruby Falls' × NC2011-1	Weeping allelism	F1(199)
'Hearts of Gold' × 'JN2'	Gold leaf allelism	F1(47)
'JN2' × 'Hearts of Gold'	Gold leaf allelism	F1(266)

[z]F_2 plants derived from separate F_1 trees.

Table 2. Segregation ratios and goodness of fit for leaf color in F_1, F_2 and backcross families derived from hybridization of *Cercis canadensis* 'Covey' × 'Forest Pansy'.[Z]

Cross	Family	Progeny phenotype		Test ratio[Y]	χ^2 (1df)	*P* value
		Green leaf	Purple leaf			
'Covey' × 'Forest Pansy'	F_1	23	0	all green		
'Covey' × 'Forest Pansy'	F_2	1230	356	3:1	5.51	0.02
('Covey' × 'Forest Pansy') × 'Covey'	BC_{1P1}	384	0	all green		
('Covey' × 'Forest Pansy') × 'Forest Pansy'	BC_{1P2}	162	156	1:1	0.05	0.82

[Z]Data from four different F_2 families combined for analysis based on test for heterogeneity (*P*=0.09).

[Y]Expected segregation based on a one gene model with purple leaf recessive to green leaf.

Table 3. Segregation ratios and goodness of fit for weeping architecture in F_1, F_2 and backcross families derived from hybridization of 'Covey' × 'Forest Pansy'.[Z]

		Progeny phenotype				
Cross	Family	Non-weeping	Weeping	Test ratio[Y]	χ^2 (1df)	*P* value
'Covey' × 'Forest Pansy'	F_1	23	0	all non-weeping		
'Covey' × 'Forest Pansy'	F_2	1209	377	3:1	1.52	0.26
'Covey' × ('Covey' x 'Forest Pansy')	BC_{1P1}	60	52	1:1	0.59	0.44
('Covey' × 'Forest Pansy') × 'Forest Pansy'	BC_{1P2}	318	0	all non-weeping		

[Z]Data from four different F_2 families combined for analysis based on test for heterogeneity (*P* =0.87).

[Y]Expected segregation based on a one gene model with weeping habit recessive to non-weeping growth habit.

Table 4. Contingency analysis to test for linkage between genes for purple leaf color and weeping architecture in F_2 families derived from hybridization of *Cercis canadensis* 'Covey' × 'Forest Pansy'.[Z]

Phenotype	Observed	Expected	χ^2 (3df)	*P* value
green, non-weeping	929	937.62	0.08	
green, weeping	301	292.38	0.25	
purple, non-weeping	280	271.38	0.27	
purple, weeping	76	84.62	0.88	
Total:	1586	1586	1.49	0.69

[Z]Data from four different F_2 families combined for analysis based on test for heterogeneity (*P*=0.63).

Table 5. Segregation ratio and goodness of fit to a dihybrid ratio for weeping and purple leaf traits in the combined F_2 family derived from hybridization of *Cercis canadensis* 'Covey' × 'Forest Pansy'.[Z]

'Covey' × 'Forest Pansy'	Progeny Phenotype				Test ratio[Y]	χ^2 (3df)	*P* value
	Non-weeping, green leaf	Weeping, green leaf	Non-weeping, purple leaf	Weeping, purple leaf			
F_2 combined:	929	301	280	76	9:3:3:1	7.97	0.05

[Z]Data from four different F_2 families combined for analysis based on test for heterogeneity (*P* =0.63).

[Y]Expected segregation based on a two gene model with purple leaf recessive to green leaf and weeping habit recessive to non-weeping habit.

Table 6. Segregation ratios and goodness of fit for individual F_2 families, combined F_2 families and backcross progeny derived from hybridization of *Cercis canadensis* 'Covey' × 'Hearts of Gold'.[Z]

Cross	Family	Progeny phenotype				Test ratio[Z]	χ^2 (1df)	*P* value
		Green	Gold	Bleached	Gold + bleached			
Covey x Hearts of Gold	F_1	37	0	0	0	all green		
Covey x Hearts of Gold #2	F_2	101	37	0	37	3:1	0.24	0.62
Covey x Hearts of Gold #3	F_2	130	30	12	42	3:1	0.03	0.86
Covey x Hearts of Gold #9	F_2	335	71	27	98	3:1	1.29	0.26

Table 6 Continued.

Covey x Hearts of Gold #12	F_2	105	24	2	26	3:1	1.85	0.17
Covey x Hearts of Gold #14	F_2	193	45	4	49	3:1	2.19	0.09
F_2 combined[y]:	F_2	864	207	45	252	3:1	3.48	0.06
Covey x (Covey x Hearts of Gold)	BC_{1P1}	152	0	0	0	all green		
Hearts of Gold x (Covey x Hearts of Gold)	BC_{1P2}	252	74	23	97	1:1	68.84	<0.001

[Z]Progeny phenotype based on leaf color, determined after approximately 30 days of growth under greenhouse conditions.

[Y]Data combined based on test for heterogeneity (P=0.55).

[X]Expected segregation based on a one gene model with (gold + bleached) recessive to green. Testing for a 3:1 ratio and 1:1 ratio (green : gold + bleached).

Table 7. Segregation ratios and goodness of fit for leaf color in F_1, F_2 bulk, and backcross families derived from hybridization of *Cercis canadensis* 'Texas White' × 'JN2'.[Z]

Cross	Family	Progeny phenotype				Test ratio[Y]	χ^2 (1df)	*P* value
		Green	Gold	Mottled	Gold + mottled			
'Texas White' × 'JN2'	F_1	463	0	0	0	all green		
'Texas White' × 'JN2' (bulk)	F_2	1025	85	85	170	3:1	73.98	<0.001
'Texas White' × ('Texas White' × 'JN2')	BC_{1P1}	877	0	0	0	all green		
'JN2' × ('Texas White' × 'JN2')	BC_{1P2}	68	23	17	40	1:1	7.26	0.007
'JN2' × 'Texas White'	F_1	8	0	0	0	all green		

[Z]Progeny phenotype based on leaf color, determined at the 2-lead stage, after approximately 30 days of growth under greenhouse conditions.

[Y]Expected segregation based on a one gene model with (gold + mottled) recessive to green. Testing for a 3:1 F_2 ratio (green : gold + mottled) and a 1:1 backcross ratio (green : gold + mottled).

Table 8. Segregation ratios and goodness of fit for leaf color in 38 individual F_2 families derived from hybridization of *Cercis canadensis* 'Texas White' × 'JN2'.[z]

F^2 family #	Progeny Phenotype				Test ratio[y]	χ^2 (1df)	P value
	Green	Gold	Mottled	Gold + mottled			
1	39	1	1	2	3:1	8.85	0.003
3	43	5	3	8	3:1	2.36	0.12
5	61	5	5	10	3:1	4.51	0.03
6	48	9	5	14	3:1	0.19	0.66
7	76	7	4	11	3:1	7.08	0.01
8	61	0	5	5	3:1	10.69	0.001
9	56	0	2	2	3:1	14.37	0.0002
10	83	0	0	0	3:1	27.67	<0.0001
11	42	8	9	17	3:1	0.46	0.5
12	54	9	2	11	3:1	2.26	0.13
13	40	0	5	5	3:1	4.63	0.03
14	41	0	10	10	3:1	0.79	0.37
15	40	0	5	5	3:1	4.63	0.03
16	60	15	0	15	3:1	1	0.32

Table 8 Continued.

17	64	11	3	14	3:1	2.07	0.15
18	38	2	2	4	3:1	5.37	0.02
19	81	21	1	22	3:1	0.73	0.39
20	31	10	0	10	3:1	0.01	0.92
21	44	1	3	4	3:1	14.67	0.02
22	54	3	0	3	3:1	11.84	0.0006
24	37	3	1	4	3:1	5.08	0.02
25	29	2	3	5	3:1	1.92	0.17
28	25	0	0	0	3:1	8.33	0.0039
29	55	0	4	4	3:1	10.45	0.0012
30	58	0	3	3	3:1	13.12	0.0003
31	62	0	0	0	3:1	20.67	<0.0001
32	43	0	10	10	3:1	1.06	0.3
33	59	0	0	0	3:1	19.67	<0.0001
34	71	0	4	4	3:1	15.47	<0.0001
36	52	0	5	5	3:1	8.01	0.0047

Table 8 Continued.

37	49	1	6	7	3:1	4.67	0.03
38	63	0	0	0	3:1	21	<0.0001
39	38	0	3	3	3:1	6.84	0.01
40	28	4	5	9	3:1	0.01	0.92
41	21	2	3	5	3:1	0.46	0.5
46	69	9	3	12	3:1	4.48	0.03
49	23	0	2	2	3:1	3.85	0.05
50	32	0	3	3	3:1	5.04	0.02

[Z]Progeny phenotype based on leaf color, determined after approximately 30 days of growth under greenhouse conditions.

[Y]Expected segregation based on a one gene model with (gold + mottled) recessive to green.

Table 9. Segregation ratios and goodness of fit for double flower in F_1 and F_2 progeny derived from hybridization and open pollination involving *Cercis canadensis* 'Flame' (double flower).

		Progeny phenotype				
Cross	Family	Double flower	Single flower	Test ratio [z]	χ^2 (1df)	*P* value
'Dwarf Alba' × 'Flame'	F_1	2	0	all double		
'Flame' (open pollinated)	OP	170	90	1:1	24.62	<0.001

[z]Expected segregation based on a one gene model with double flower dominant to single flower.

Table 10. Segregation ratios for leaf variegation in F_1 families derived from hybridizations of *Cercis canadensis* 'Floating Clouds' with four other parents.

Cross	Family	Progeny phenotype	
		Variegated	Green
'Covey' × 'Floating Clouds'	F_1	0	41
'Floating Clouds' × 'Covey'	F_1	5	0
'Floating Clouds' × 'Texas White'	F_1	127	0
'Floating Clouds' × 'Rising Sun'	F_1	61	0
'Floating Clouds' × NC2006-14	F_1	15	0

Table 11. Segregation ratios and goodness of fit for leaf variegation in progeny derived from hybridizations of *Cercis canadensis* 'Covey' × 'Silver Cloud'.

Cross	Family	Progeny phenotype		Test ratio[Z]	χ^2 (1df)	*P* value
		Green	Variegated			
'Covey' × 'Silver Cloud'	F_1	2	0	all green		
'Covey' × 'Silver Cloud' (99-18-1)	F_2	1259	365	3:1	5.52	0.02
'Covey' × 'Silver Cloud' (99-18-2)	F_2	112	35	3:1	0.11	0.74

[Z]Expected segregation based on a one gene model with variegated recessive to green.

Table 12. Progeny phenotypes derived from hybridization of *Cercis canadensis* 'Ruby Falls' × 'Greswan', and open pollination of 'Greswan'.

		Progeny phenotype:	
Cross	Family	Purple	Green
'Greswan'	OP	3	88
'Ruby Falls' × 'Greswan'	F_1	195	1

Table 13. Progeny phenotypes derived from reciprocal crosses of *Cercis canadensis* 'Hearts of Gold' and 'JN2', characterized by cotyledon color and leaf color. [Z,Y]

Phenotypes	Hearts of Gold x JN2	JN2 x Hearts of Gold
Green	(11) 8	(48) 61
Gold	(19) 16	(100) 87
Chartreuse [X]	(15) 19	(68) 102
Bleached[W]	(16) 4	(88) 16
Total progeny[V]	(61) 47	(304) 266

[Z] Values within parentheses indicate number of progeny based on cotyledon color.

[Y] Values to the right of parentheses indicate final number of progeny based on leaf color, determined after about 90 days of growth under greenhouse conditions.

[X] Values at three months are greater than those originally recorded in the cotyledon stage due to bleached progeny developing into the chartreuse phenotype.

[W] Values at three months are lower than those originally recorded at the cotyledon stage due to lethality of bleached seedlings and reclassification of some bleached seedlings as chartreuse.

[V] Values for total progeny, in parentheses, are less than those originally recorded due to lethality of bleached and chartreuse progeny.

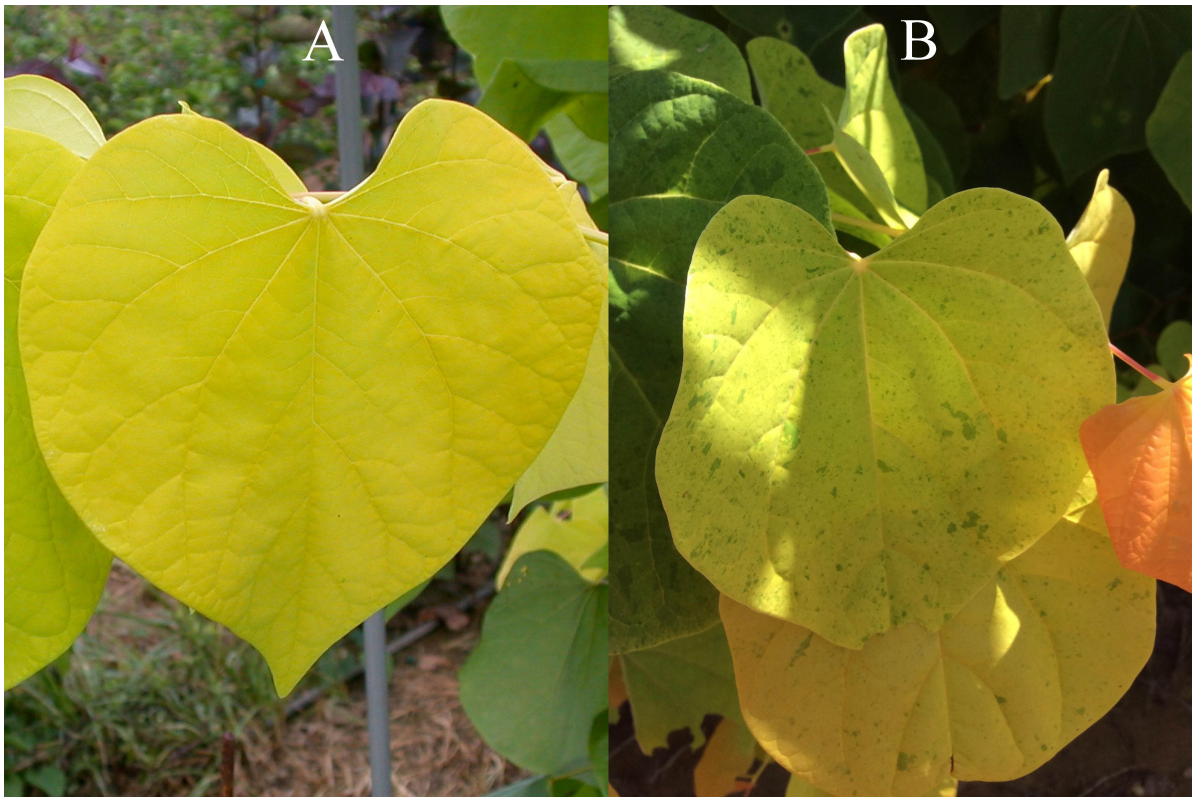

Figure 1. Foliage of *Cercis canadensis* A) ‘Hearts of Gold’ and B) ‘JN2’ showing solid gold color in ‘Hearts of Gold’ and the gold with green spots phenotype of ‘JN2’.

Figure 2. Double (left) vs. single flower of *Cercis canadensis*.

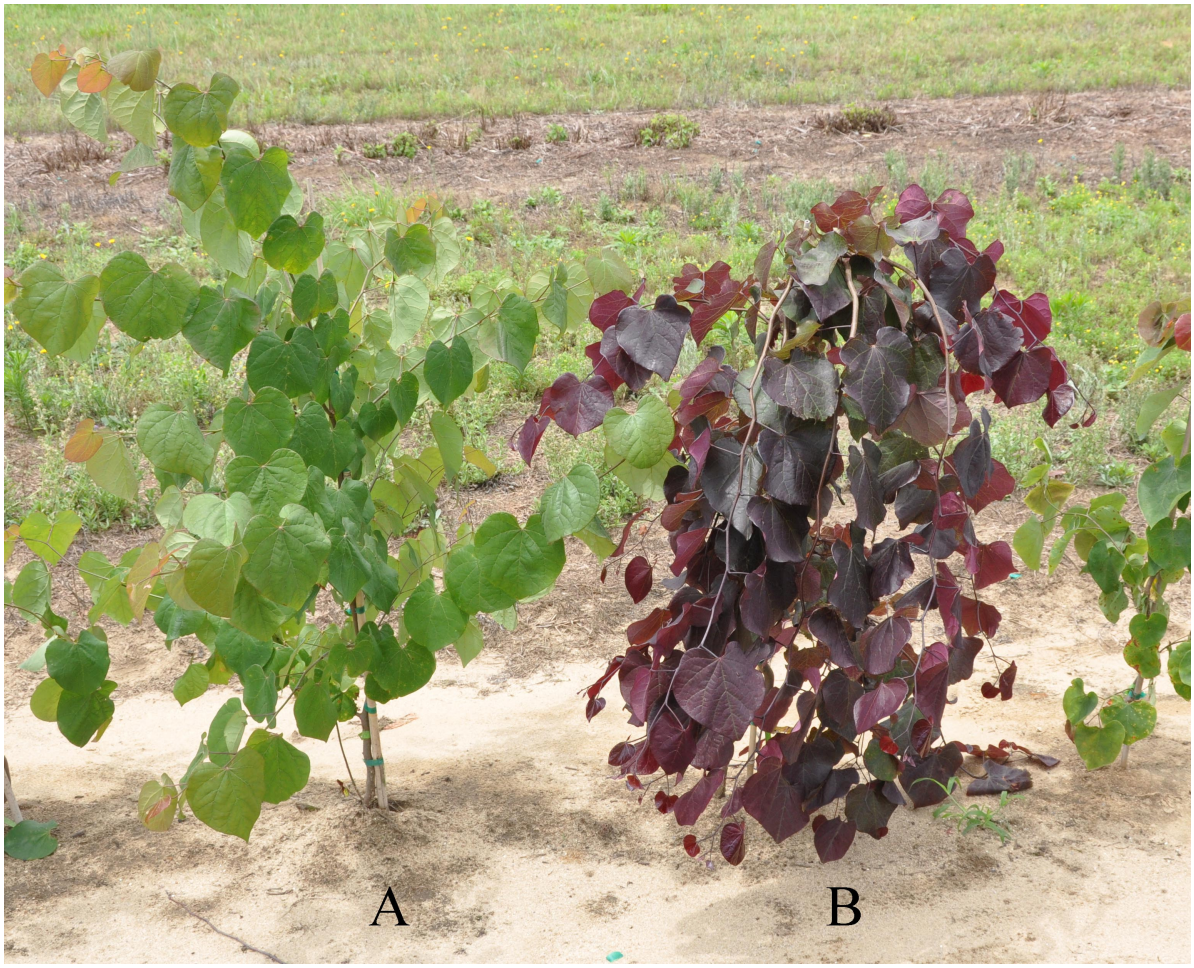

Figure 3. *Cercis canadensis* 'Covey' × 'Forest Pansy' F_2 trees exhibiting segregation for: A) green leaf, non-weeping phenotype, B) purple leaf, weeping phenotype.

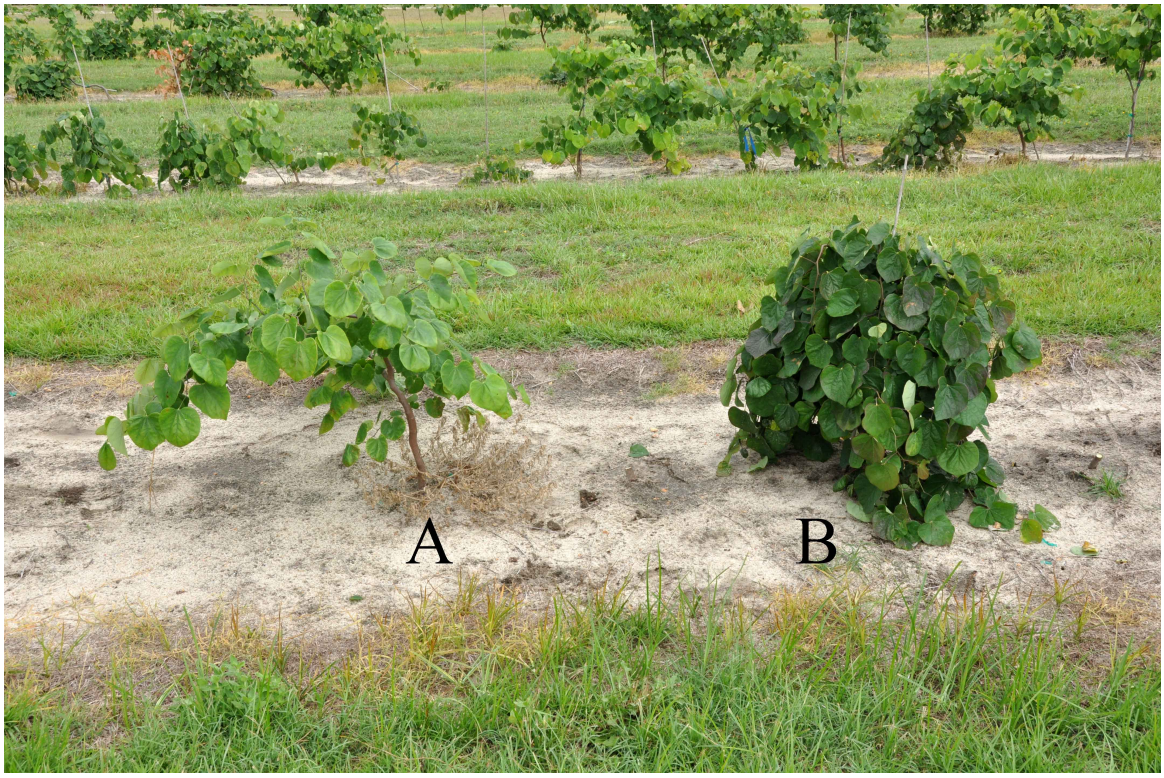

Figure 4. *Cercis canadensis* ‘Covey’ × ‘Forest Pansy’ segregating progeny exhibiting: A) semi-pendulous habit (scored as non-weeping) and B) weeping habit.

Figure 5. Bleached progeny derived from *Cercis canadensis* 'Covey' × 'Hearts of Gold', approximately one month old and demonstrating chlorophyll deficiency. The majority of bleached seedlings were lethal.

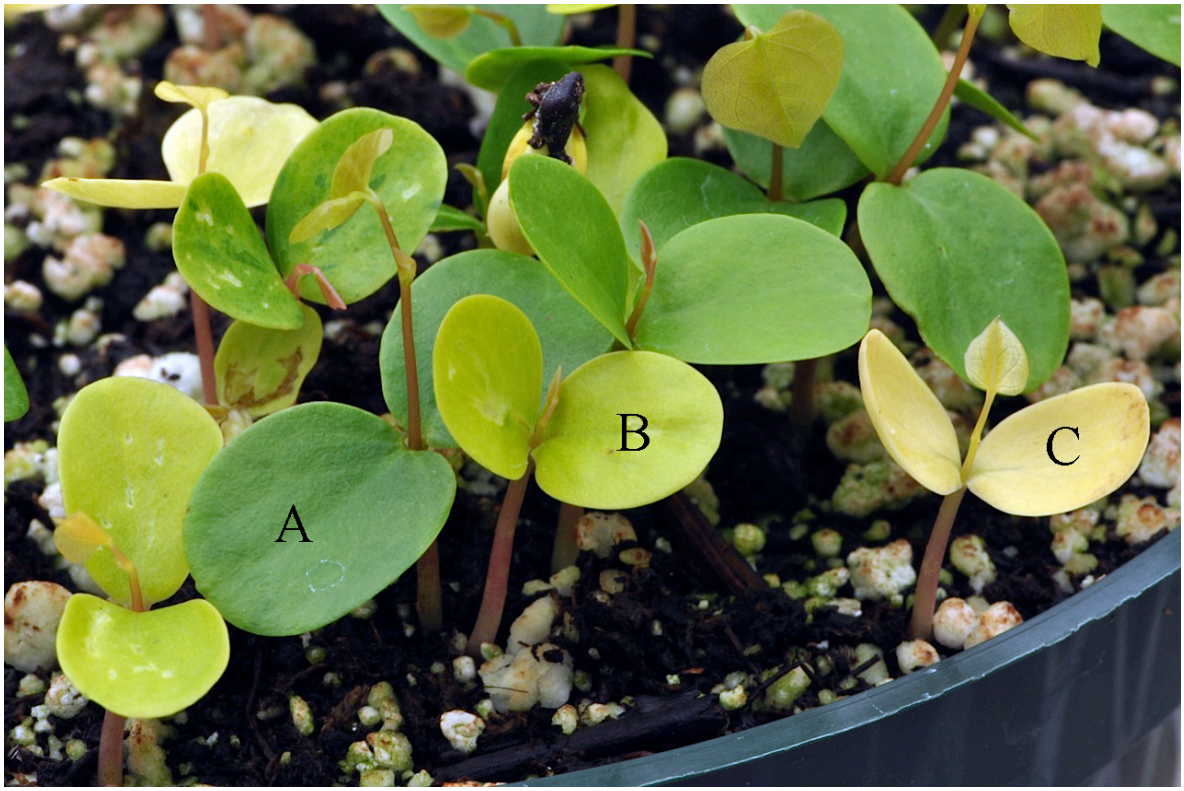

Figure 6. Examples of progeny phenotypes derived from *Cercis canadensis* 'Covey' × 'Hearts of Gold' segregating for cotyledon color, classified as: a) green cotyledons, b) gold cotyledons, c) bleached cotyledons.

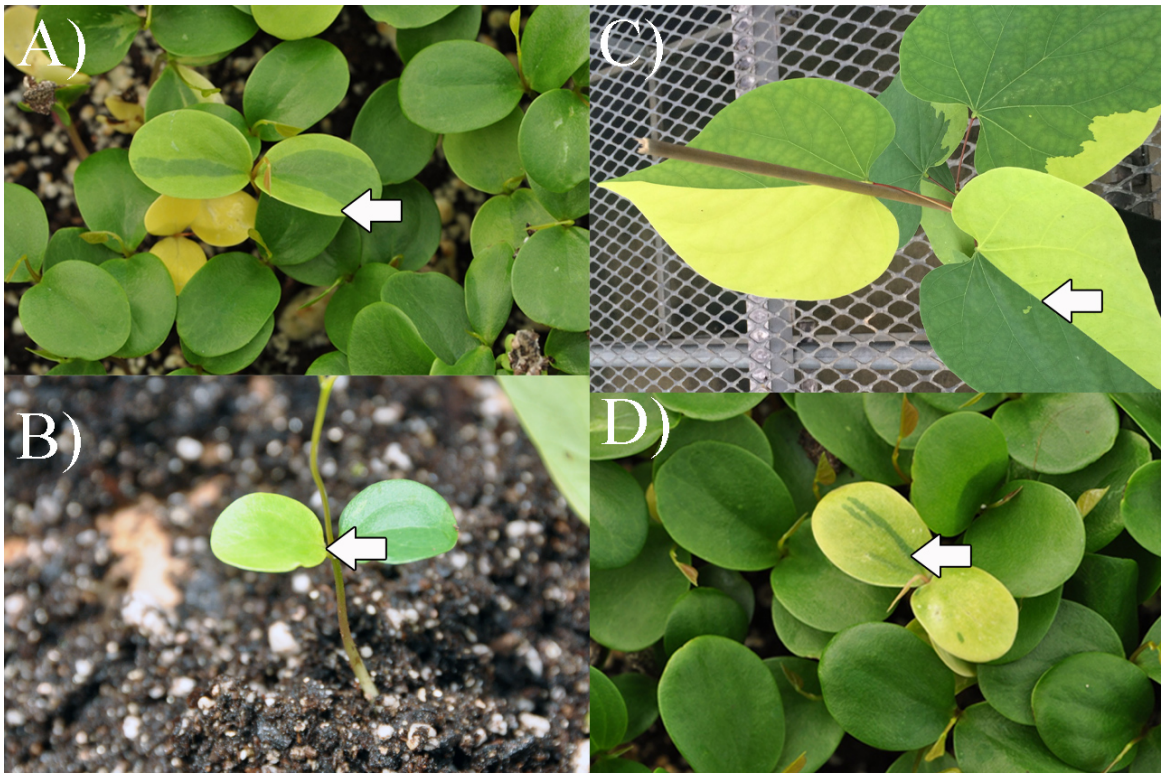

Figure 7. Progeny derived from *Cercis canadensis* hybridizations involving 'Hearts of Gold', demonstrating putative transposon mediated variegation. A) 'Covey' × 'Hearts of Gold' F_2 showing a green cotyledon with gold variegation. B) 'Covey' × 'Hearts of Gold' F_2 showing both a green and gold cotyledon. C) 'Covey' × 'Hearts of Gold' F_2, 4 months old showing green-gold leaf variegation on opposite sides of the midrib. D) 'Covey' × 'Hearts of Gold' F_1 showing gold leaf cotyledon with green variegation. Arrows indicate variegated sectors.

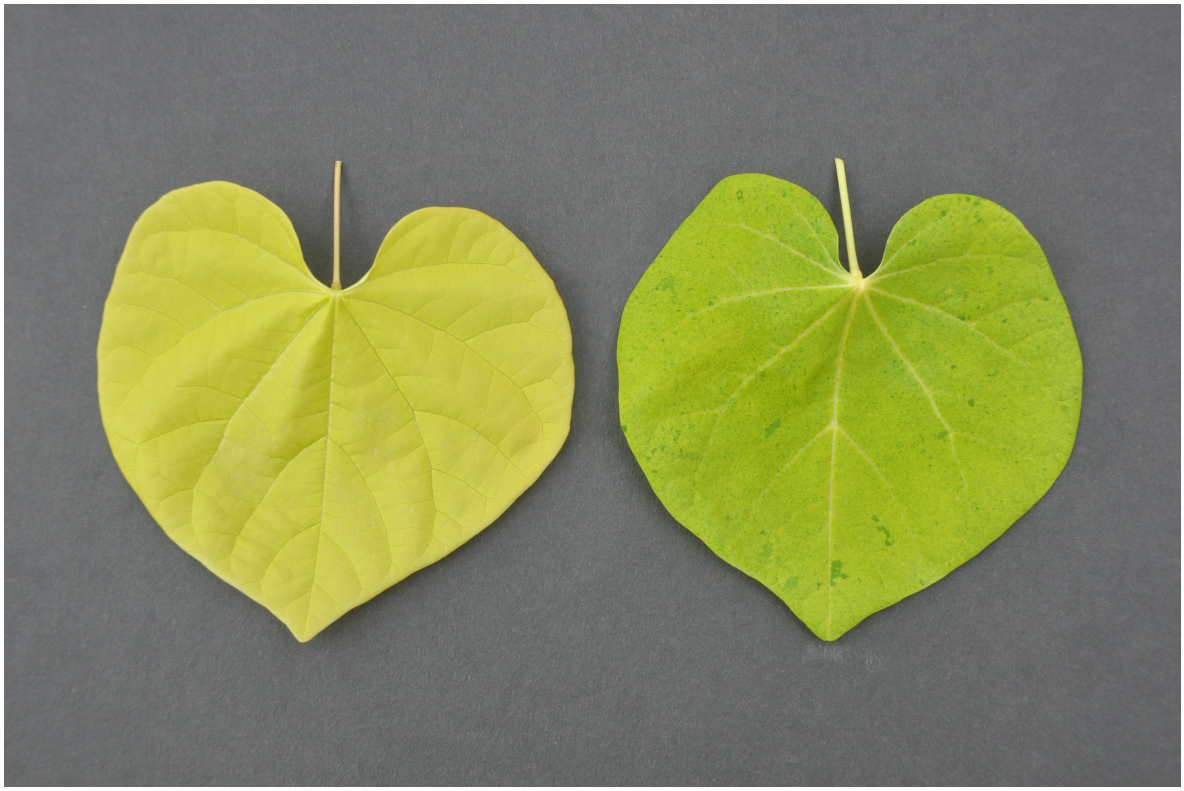

Figure 8. Comparison of solid gold leaf phenotype and mottled leaf phenotype recovered in the F_2 of *Cercis canadensis* 'Texas White' × 'JN2'.

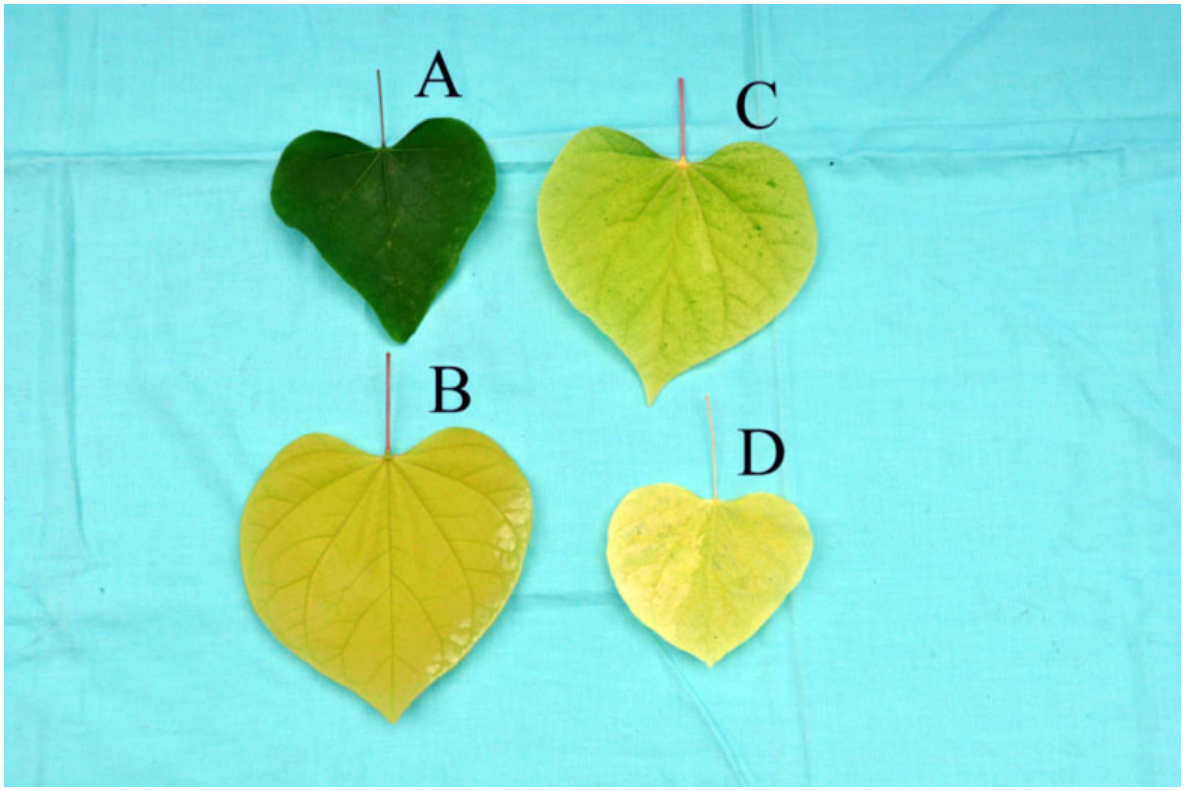

Figure 9. Examples of phenotypic categories established for mature leaves in progeny derived from the hybridization of *Cercis canadensis* 'Hearts of Gold' × 'JN2' and the reciprocal: A) Wild-type green, B) gold, C) chartreuse, D) bleached.