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Are 'Barton' and 'Cloud 9' the Same Cultivar of *Cornus florida* L.?[1]

Mark T. Windham[2] and Robert N. Trigiano[3]
Tennessee Agricultural Experiment Station
The University of Tennessee, Knoxville, TN 37901-1071

Abstract

Ten flowering dogwood (*Cornus florida*) cultivars were evaluated for phenotypic characters including resistance to spot anthracnose and dogwood canker, susceptibility to frost damage, leaf color, bract length and color, bloom number and reproductive phenology. Cultivars differed by at least three phenotypic characters except for 'Barton' and 'Cloud 9', which when compared with each other, were not significantly different at any characteristic. DNA amplification fingerprinting (DAF) using 17 octamer oligonucleotide primers could not distinguish these cultivars. Furthermore, arbitrary signatures from amplification profiles (ASAP), a technique that produces 'fingerprints of fingerprints', which is capable of detecting more differences than DAF, also failed to reveal any polymorphic DNA. Therefore, based on the lack of phenotypic and genetic differences between these two cultivars, we conclude that they are the same genotype.

Index words: genetic analysis, fingerprinting, cultivar identification.

Species used in this study: flowering dogwood (*Cornus florida* L.).

Significance to Nursery Industry

Flowering dogwood (*Cornus florida* L.) is a valuable landscape tree that is popular in the nursery industry. Using phenotypic characters, eight of ten dogwood cultivars could easily be separated from each other. However, neither phenotypic or genetic analysis (DNA fingerprinting) could separate 'Barton' and 'Cloud 9'. In recent studies, DNA fingerprinting has been used successfully to separate closely related genotypes of dogwoods, including half-sibs, and will prove useful in protecting the integrity of patented and trademarked plant material.

Introduction

The flowering dogwood, *Cornus florida* L, is a small, flowering tree that is very important to the commercial nursery industry, and more than 100 cultivars of this species have been named (7, 10). However, less than twenty of these cultivars are currently popular in the nursery trade (9). Most cultivars can be segregated into groups based on bract or foliage color. Cultivars can also be distinguished based on growth habit (size, upright stature, weeping, etc.), color of bark and fruit, size of bracts, time of flowering, growth rate (fast, slow, dwarf, etc.), and disease or insect resistance.

'Barton', selected by Marvin Barton, Birmingham, AL, in 1956 (10) and 'Cloud 9' (Plant Patent No. 12112) introduced by Hubert Chase, Chase, AL, in 1961 (10) are two white, early flowering dogwood cultivars that have been difficult to distinguish based on growth habit, bract color and size, foliage color, etc. (Don Shadow, Shadow Nursery, Winchester, TN, personal communication). Although both cultivars have been very popular in the past, only 'Cloud 9' remains very common in the nursery trade. In the Tennessee Nursery Buyers Guide (9), 17 of 56 nurseries that are listed as producing white flowering dogwoods sell the cultivar 'Cloud 9'. Only one of those nurseries continues to carry the cultivar 'Barton' in its catalog (Shadow Nursery, Winchester, TN).

To determine which phenotypic characters would be useful in distinguishing cultivars of similar appearance, a flowering dogwood cultivar trial was established at the Plateau Experiment Station, Crossville, TN. This trial contained ten cultivars that have been popular in the nursery trade for many years. This research report attempts to distinguish dogwood cultivars based on phenotypic characters and genetic studies including genomic analysis using DNA amplification fingerprinting (DAF) (2) and arbitrary signatures from amplification profiles (ASAP) (4).

Materials and Methods

Plant material. Trees of ten flowering dogwood cultivars ('Barton', 'Cherokee Princess', 'Cloud 9', 'First Lady', 'Fragrant Cloud', 'Plena', 'Purple Glory', 'Rubra', 'Springtime', and 'Welch's Jr. Miss') were obtained from a commercial wholesale nursery in Tennessee and transplanted into a field at the Plateau Experiment Station, Crossville, TN, in 1986. At transplanting, trees had been budded for approximately 19 months. Trees were spaced 1.3 m apart within rows and rows were spaced 3 m apart. Trees were irrigated when necessary during the first year after transplanting. Trees were fertilized each year based on recommendations from soil tests and weed control was obtain by spraying weeds at the base of trees with Round Up® (Monsanto, St. Louis, MO) and mowing alleys between rows.

Assessment of phenotypic characters. Trees of the ten cultivars were evaluated for bract color (pink or white) , foliage color (green, varigated, or purple), susceptibility of blooms to frost damage and spot anthracnose, caused by *Elsinoe corni* Jenk. Bitancourt, susceptibility of tree trunks to dogwood canker (etiology unknown), bloom number, bract length, and reproduction phenology three years after transplanting. Frost damage was assessed using the following scale: 0 = no damage, 1 = slight scorch at bract edges, 2 = few bracts with

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[2]Associate Professor, Entomology and Plant Pathology.

[3]Professor, Ornamental Horticulture and Landscape Design.

scorch extending from edges into middle of bracts, 3 = most bracts with scorch extending from edges into middle of bracts, 4 = all bracts with damage beyond bract edges and 5 = bracts destroyed. Spot anthracnose susceptibility of blooms was estimated using the following scale: 0 = healthy, 1 = 1–5 lesions/bract, 2 = 5–10 lesions/bract, 3 = 5–10 lesions (with necrotic centers)/bract, 4 = >10 lesions (with necrotic centers)/bract and 5 = bracts distorted and destroyed. Trees were evaluated for dogwood canker by examining the trunks for canker like depressions in internode regions as described by Lambe and Wills (8). Bract length was recorded by randomly selecting five blooms from each tree and measuring the longest pair of bracts (typically the inner pair of bracts subtending the inflorescence). Number of blooms was determined by counting all blooms on each tree. Reproduction phenology was estimated every 7 days for three weeks during the trees' blooming period by the following scale: 0 = flower bud closed, 1 = buds swollen, bracts without color, 2 = bracts with color, but buds not completely open, 3 = buds completely open, 4 = bracts fully expanded, 5 = less than half of flowers with anthers dehisced, 6 = more than half of flowers with anthers dehisced, 7 = bracts beginning to drop off, 8 = less than half of bracts remaining, 9 = all bracts dropped, ovaries beginning to swell and 10 = fruit developing.

Statistical analysis of phenotypic characters. Trees were arranged in a randomized complete block design with five replicates. Each replicate consisted of three trees. Data were analyzed using the GLM procedure of the Statistical Analysis Systems (11). Differences in the measured variables were determined by an F test when significant F values were detected, means were separated by the Student-Newman-Keul Test (p = 0.05).

Genetic analysis of cultivars. Young leaves from three individual trees of 'Barton' obtained from Tennessee Valley Nursery, Winchester, TN, and Byers Nursery, Huntsville, AL, and 'Cloud 9' obtained from Tennessee Valley Nursery, and Commercial Nursery, Decherd, TN, and samples from trees of the two cultivars used in the phenotypic study at Crossville, TN, were collected in the early morning, packed on ice for return to the laboratory, and stored at –70C until needed. Genomic DNA was extracted from 25–50 mg of tissue using a commercially available kit (Puregene; Gentra Systems, Inc., Minneapolis, MN). DAF analysis of genomic DNA was completed according to the method of Caetano-Anollés et al. (2). Reactions were assembled in a total volume of 20 µl containing 3 µM primer, 1.5 mM $MgCl_2$, 200 µM dNTP, 8 units of Stoffel fragment DNA polymerase, and 0.1 ng/µl template DNA and amplified in a thermocycler (Easy Cycler Twin Block System; Ericomp, Inc., San Diego, CA) for 35 cycles each consisting of 10 sec steps at 96C and 30C. Seventeen standard (nonstructured) octamer primers were used and had the following (5'–3') sequences: GAGCCTGT, CCTGTGAG, AACGGGTG, GTAACGGC, GACGTAGG, GATCGCAG, CTAACGCC, GAAACGCC, GTTACGCC, GTATCGCC , AATGCAGC, CCGAGCTG, CCTGGTGG, CGTGGTGG, CCTGCTGG, CAGCTCGG, and CGCGGCCA. DAF products generated with primers AACGGGTG, GTAACGGC and GAAACGCC were diluted 1:49 with sterile, distilled water and used as template in ASAP reactions (3). ASAP cocktails were assembled as above except that primer and $MgSO_4$ concentrations were 9 µM and 4 mM, respectively, and TTNK10 buffer (4) was used instead of Stoffel buffer. Each of the 4 mini-hairpin decamer primers contained at their 5' termini the constant GCCGAAGC sequence (H) and had one of the following arbitrary 3' nucleotide sequences: GCA, GGT, CAT and CTA. Thirty-five cycles of 30 sec each at 96C, 30C, and 72C were used for the amplifications. Twelve ASAP profiles were generated using the products of 3 DAF reactions as templates for each of the four mini-hairpin primers. All DAF and ASAP reactions were repeated at least twice.

DAF and ASAP products were diluted 1:1 with sterile, distilled water and separated electrophoretically on 10% polyacrylamide gels backed on polyester film (Gelbond; FMC, Corp., Rockland, ME). DNA was visualized using a fast and sensitive silver staining method capable of detecting picogram quantities of DNA (1). Silver stained gels were viewed on a light box and bands of 50–750 base pairs in length were scored as either present or absent.

Results and Discussion

Phenotypic character separations. Using the phenotypic characters described above, cultivars differed for 0–8 characters (Table 1). All cultivars were distinguishable from each other by at least three characters except for 'Barton' and 'Cloud 9', which did not statistically differ from each other for any phenotypic characters evaluated (Table 1).

Table 1. Comparison of phenotypic characters of five-year-old (from budding) trees of ten flowering dogwood cultivars grown at the Plateau Experiment Station, Crossville, TN.

Cultivar	Bract color	Leaf color	Frost damage	Canker incidence	Spot anthracnose	Bloom number	Bract length	Phenology		
								April 19	April 26	May 3
Barton	W	G	2.0a[z]	0c	3.4a	70b	3.4c	5a	6a	7a
Cherokee Princess	W	G	1.8b	0c	0.8cd	62b	5.0a	3c	6a	7a
Cloud 9	W	G	2.4a	5bc	3.2a	72b	3.4c	5a	6a	8a
First Lady	W	V	0.5b	0c	0.3d	3e	2.7cd	1d	5a	7a
Fragrant Cloud	W	G	0.5b	12b	1.0c	46cd	4.4ab	3c	6a	6b
Plena	W	G	0.0c	17b	0.0e	91a	3.4c	1d	3b	4c
Purple Glory	P	Pp	0.2bc	63a	1.2c	27cd	3.4c	2cd	6a	6b
Rubra	P	G	0.0c	0c	1.8b	58b	3.5bc	3c	6a	6b
Spring Time	W	G	0.0c	10b	0.4d	50bc	4.1b	4b	6a	6b
Welch's Junior Miss	P	G	0.0c	5bc	1.8b	17d	2.0d	2bc	6a	6b

[z]Means followed by the same letter do not differ according to Student-Newman-Keul Test (p = 0.05), n = 5.

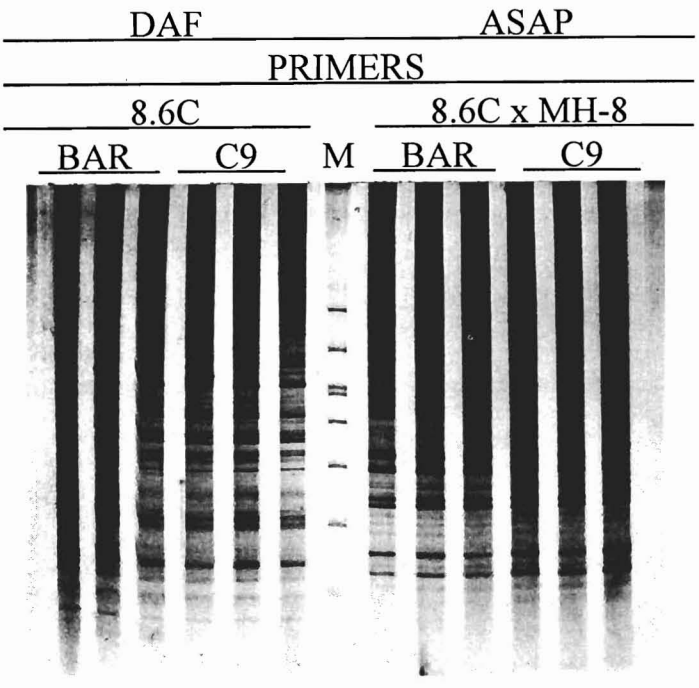

Fig. 1. DNA amplification fingerprinting (DAF) and Arbitrary Signatures of Amplified Profiles (ASAP) of three individuals of 'Barton' (BAR) and 'Cloud 9' (C9). The six lanes on the left represent monomorphic DAF profiles of both cultivars generated by primer 8.6 (AACGGGTG). The middle single lane contains molecular markers in base pairs (1000, 700, 525,500, 400, 300, 200, and 100). The six lanes on the right are ASAP profiles of both cultivars produced by the combination of 8.6c reamplified with MH-8 (GCGAAGCCAT). All character loci are monomorphic.

The lack of phenotypic differences between 'Barton' and 'Cloud 9' may be one reason why the popularity of 'Barton' has waned over the last decade. Although very similar in morphological and growth characteristics, questions concerning genetic relationships between these two cultivars have remained unanswered for many years because of the lack of technology for analyzing portions of their genome. The technology is now available.

Genetic comparison of 'Barton' and 'Cloud 9'. Genomic analysis techniques such as DAF have been successfully used to delineate closely related cultivars of dogwoods (14). Using these techniques, unique banding patterns ('bar codes') for cultivars can be recorded on polyacrylamide gel and used to identify individual cultivars or unique individuals. Conversely, this technique is useful in determining when two cultivars are extremely similar or the same genotypes. DAF fingerprints of 'Barton' and 'Cloud 9' obtained with standard octamer primers were clear and reproducible (Figure 1). However, the primers failed to detect any polymorphic regions of DNA between the two cultivars; all 486 character loci were monomorphic or identical. In a related study of these and other dogwood cultivars, one polymorphism was discovered between 'Barton' and 'Cloud 9' whereas numerous polymorphisms were found between the other cultivars including 'Springtime' and 'Cherokee Princess' (unpublished data). Complete homogeneity between these two cultivars, even if closely related, is surprising since DAF has been used to distinguish somatic mutations of chrysanthemum cultivars (12) and isolates of *Discula destructiva* Redlin, the cause of dogwood anthracnose (13). Since the ASAP technique is capable of detecting increased levels of polymorphic DNA not revealed by other less discerning fingerprinting techniques, it has been used to discover the identities of recalcitrant individuals such as 'Barton' and 'Cloud 9' that defy genomic analysis (5, 6, 15). However, all of the 293 character loci uncovered by the ASAP technique were monomorphic between the two cultivars (Figure 1). Therefore, genomic analysis by DNA fingerprinting tecniques supports the contention that the two cultivars are the same genotype.

Since cultivars 'Barton' and 'Cloud 9' were not distinguishable using phenotypic characters and genetic analysis, we conclude that 'Barton' and 'Cloud 9' are two names for the same genotype of flowering dogwood. How clones of the same tree came to be produced as two different cultivars is unknown. Our hypothesis is that these cultivars were actually two distinct, but very similar, cultivars of flowering dogwood when they were introduced. However, due to phenotypic similarities, the two cultivars were confused in the industry, one of the cultivars was excluded and eventually the genetic material was lost. However, both names continued to be used in the nursery industry.

Analysis of genomic DNA of flowering dogwoods and other ornamental plants has become an important tool for protecting the integrity of cultivars and other patented plant materials. We anticipate that the usage of this type of technology to separate closely related phenotypes in the woody ornamental industry will become more routine in the near future.

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Influence of Storage Temperatures on Long-term Seed Viability of Selected Native Ericaceous Species[1]

Christopher T. Glenn[2], Frank A. Blazich[3], and Stuart L. Warren[3]
Department of Horticultural Science
North Carolina State University, Raleigh, NC 27695-7609

Abstract

Following harvest of capsules, drying, and seed extraction, seeds of *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum (drooping leucothoe), *Rhododendron carolinianum* Rehd. (Carolina rhododendron), *Rhododendron catawbiense* Michx. (Catawba rhododendron), and *Rhododendron maximum* L. (rosebay rhododendron) were stored for 0, 1, 2, 3, 4 or 5 years at –18, 4 or 23C (0, 39 or 73F) and then germinated at 25C (77F) or an 8/16 hr thermoperiod of 25/15C (77/59F) with daily photoperiods of 0, 1 or 24 hr. Storage at –18 or 4C (0 or 39F) were most effective for maintaining seed viability of all species. After 5 years storage at –18 or 4C (0 or 39F), viability of *L. fontanesiana*, *R. catawbiense*, and *R. maximum* was relatively unchanged with total germination of 59%, 87%, and 88%, respectively. The same was noted for seeds of *K. latifolia* and *R. carolinianum* with total germination of 77% and 91%, respectively, after storage for 4 years at the same temperatures. Storage at 23C (73F) was the least effective for maintaining viability. After storage for 1 year at 23C (73F), germination decreased significantly for all species except *R. carolinianum*. By year 3, storage at 23C (73F) reduced seed viability of *L. fontanesiana* to essentially zero. The same occurred by year 4 for seeds of *R. catawbiense* and *R. maximum* stored at 23C (73F). Viability of *K. latifolia* also decreased under storage at 23C (73F) with germination of 14% noted by year 4. Viability of *R. carolinianum* did not decrease as rapidly as the other species when stored at 23C (73F) with total germination of 77% occurring by year 4. Regardless of storage duration, the photoperiod and temperature requirements for maximum germination of all species did not change.

Index words: sexual propagation, *Kalmia latifolia, Leucothoe fontanesiana, Rhododendron carolinianum, Rhododendron catawbiense, Rhododendron maximum*, native plants.

Significance to the Nursery Industry

Seed viability of *Kalmia latifolia* (mountain laurel), *Leucothoe fontanesiana* (drooping leucothoe), *Rhododendron carolinianum* (Carolina rhododendron), *Rhododendron catawbiense* (Catawba rhododendron), and *Rhododendron maximum* (rosebay rhododendron) can be maintained relatively constant for 4 to 5 years when seeds are dried to moisture contents of 4% to 7% and the seeds stored in sealed containers under freezer [–18C (0F)] or refrigerated [4C (39F)] conditions. For all species, except *R. carolinianum*, room temperature storage [23C (73F)] should be avoided as viability is lost rapidly. Lack of change in seed viability following storage for 4 to 5 years at –18 or 4C (0 or 39F) suggests these storage conditions should permit maintenance of viability for periods greatly exceeding these lengths of time. Results also demonstrated that the photoperiod and temperature requirements for maximum germination of all species remained constant; they did not change with storage duration.

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[2]Graduate Research Assistant.

[3]Professor.

Introduction

Many woody ericaceous species native to the Appalachian Mountains of the United States are desirable landscape plants, including *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum (drooping leucothoe), *Rhododendron carolinianum* Rehd. (Carolina rhododendron),