This article was listed in Forest Nursery Notes, Summer 2007

44. AFLP markers identify *Cornus florida* cultivars and lines. Smith, N. R., Trigiano, R. N., Windham, M. T., Lamour, K. H., Finley, L. S., Wang, X., and Rinehart, T. A. Journal of the American Society for Horticultural Science 132(1):90-96. 2007.

J. Amer. Soc. Hort. Sci. 132(1):90–96. 2007.

AFLP Markers Identify *Cornus florida* Cultivars and Lines

Naomi R. Smith[1], Robert N. Trigiano[2], Mark T. Windham, Kurt H. Lamour, Ledare S. Finley, and Xinwang Wang
Department of Entomology and Plant Pathology, University of Tennessee, 205 Ellington Hall, 2431 Joe Johnson Drive, Knoxville, TN 37996-4560

Timothy A. Rinehart
USDA/ARS, Thad Cochran Mid-South Horticultural Research Center, P.O. Box 287, Poplarville, MS 39470

Additional index words. cluster analysis, dichotomous key, flowering dogwood, genetic variability, molecular markers

Abstract. **Flowering dogwood (*Cornus florida* L.) is an important tree of forests and urban landscapes in the eastern United States. Amplified fragment length polymorphism (AFLP) markers were generated from genomic DNA of 17 cultivars and lines, and four duplicate samples of selective cultivars. Specific markers were identified for all except the following two lines and cultivar: MW94-67, MW95-12, and 'Plena'. A dichotomous cultivar identification key was constructed based on AFLP data, and specific peaks or combinations of peaks were identified for all cultivars and lines. The key was assessed with seven anonymous (unlabeled) dogwood samples, and all unknowns except one were identified using the dichotomous key. Two of the unknown samples, 'Cherokee Chief' and 'Cherokee Brave', were difficult to distinguish using the AFLP markers. Intracultivar variation, up to 36% dissimilarity, was observed between duplicate samples of the same cultivar from different trees, suggesting that some mislabeling of trees had occurred at the nursery. The cultivar-specific AFLP markers can be used in breeding applications, patent protection, and in future projects, such as mapping the *C. florida* genome.**

Flowering dogwood is an important tree of forests and urban landscapes in the eastern United States. This native tree blooms early in the spring as new leaves unfold. The inflorescence consists of petallike subtending bracts of red, pink, or white surrounding a cluster of 20 or more tiny true flowers that are yellow or white (Witte et al., 2000). Dogwood is an attractive tree in all seasons and has become a widely used landscape tree. Although flowering dogwood is a native tree and many wild trees exist, the popularity of this tree has led to the development of many cultivars. New cultivars are either developed from mutations or sports of other cultivars, or from the selection of wild dogwood trees for various horticultural traits (Witte et al., 2000). Cultivated lines of dogwoods are propagated from axillary buds, which are grafted onto rootstocks that are produced from wild (native) seed collections (Dirr, 1998). This propagation technique produces cultivars or lines of purportedly identical trees with specific, desirable phenotypic traits. Cultivated selections have been developed for large bracts; double bracts; red, pink, or white bracts; variegated leaves; various growth habits; and disease resistance. There are currently more than 80 cultivars of flowering dogwood (Witte et al., 2000) and many are described and illustrated in Cappiello and Shadow (2005). Because many cultivars are very similar in appearance, there is a need for a method of identification that is based on genotype rather than solely on phenotypic characteristics.

Received for publication 22 June 2006. Accepted for publication 28 Sept. 2006. Financial support provided by USDA/ARS agreement no. 58-6404-2-0057 and the Tennessee Agricultural Expt. Sta.
[1]Current address: Western Kentucky University, Biology Department, 1 Big Red Way, TCNW 204, Bowling Green, KY 42101.
[2]Corresponding author: Department of Entomology and Plant Pathology, University of Tennessee, 205 Ellington Hall, 2431 Joe Johnson Drive, Knoxville. E-mail: rtrigian@utk.edu

Molecular markers can be very useful in cultivar identification or in determination of parentage of sexually propagated species. This is particularly important for proprietary plants, because molecular markers can be used in patent applications and subsequently for protection of the patented cultivars against infringement (Saunders et al., 2001; Weising et al., 1995). Currently, one of the most popular DNA fingerprinting techniques is amplified fragment length polymorphism (AFLP) (Vos et al., 1995). Amplified fragment length polymorphisms are widely used because they can be generated without prior knowledge of an organism's genome and are generally reproducible within and between laboratories (Amador et al., 2001; Saunders et al., 2001; Savelkoul et al., 1999; Vos and Kuiper, 1997).

In this study, AFLPs were generated to assess the genetic variability between selected cultivars and lines of *Cornus florida* and to construct a dichotomous key using specific molecular markers to distinguish some of the more popular cultivars and breeding lines from one another.

Materials and Methods

Emerging leaves from 17 flowering dogwood cultivars and lines were collected early in the morning to reduce interfering compounds such as phenols and carbohydrates, and were immediately frozen at –80 °C. Leaves from two different trees of four cultivars were collected from different locations, providing duplicate samples for these cultivars (Table 1). Genomic DNA was isolated from dogwood leaves from each cultivar, including each tree of the duplicated samples, according to directions provided for the Qiagen DNeasy Plant DNA isolation kit (Qiagen, Valencia, Calif.). DNA was isolated twice from both trees representing 'Cherokee Sunset', 'Cherokee Chief', 'Cherokee Brave', and 'Cloud 9', and AFLPs generated using the DNA from the independent isolations.

Table 1. *Cornus florida* cultivars and unnamed lines included in the amplified fragment length polymorphism analysis.

Cultivars	Lines
Appalachian Blush	MW 95-4
Appalachian Mist	MW 95-12
Appalachian Snow	MW 95-28
Appalachian Spring	MW 94-60
Cherokee Brave	MW 94-67
Cherokee Chief[z]	
Cherokee Daybreak[z]	
Cherokee Princess	
Cherokee Sunset[z]	
Cloud 9[z]	
Fragrant Cloud	
Plena	

[z]Duplicate sample was also evaluated.

The AFLP method used in this study was a modification of the original protocol described by Vos et al. (1995). Sequences of adaptors and primers were those described by Amador et al. (2001). Genomic DNA was digested with *Eco* RI and *Mse* I restriction enzymes, and a preselective amplification using primer combination Eco + A and Mse + C was performed. Selective amplifications were completed with 12 nonfluorescent Eco + ANN and Mse + CNN (N = A, T, G, or C) primer combinations. Fluorescent labeling of amplicons was accomplished using the protocol described by Habera et al. (2004), which involved an additional amplification step that labels selective amplicons with a Proligo WellRED fluorescent Eco + A primer (Sigma-Aldrich, St. Louis). This polymerase chain reaction did not amplify any new products, but simply labeled all amplicons from the selective reactions.

Labeled fragments were visualized using a Beckman-Coulter CEQ 8000 Genetic Analysis System (Beckman-Coulter, Fullerton, Calif.). An electropherogram, based on fragment size, was produced for each sample. A 600-bp size standard from Beckman was included in each sample well. The Frag-4 CEQ standard program was slightly modified by increasing separation time from 60 to 75 min, which produced fingerprints with well-spaced fragments. All data were reported as binary (1, peak present; 0, peak absent) and were manually compared with the original electropherograms to verify presence or absence of peaks. Manual corrections were conservative so that analyses were not based on weak peaks or peaks that may have been background noise, generally less than 15,000 relative fluorescent units (Saunders et al., 2001). Peaks that were observed in only one cultivar were noted and used as markers that uniquely identified individual dogwood cultivars or lines.

Verified binary data from 12 primer pairs were analyzed using NTSYSpc (version 2.02g; Exeter Software, Setauket, N.Y.) to estimate the genetic distance or similarity between all the samples. A cluster analysis was performed using the unweighted pair group cluster analysis using arithmetic means method with the Jaccard coefficient and was visualized with a genetic distance tree. Bootstrap values were calculated for the genetic distance tree with Phylogenetic Analysis Using Parsimony (version 4.0b10 for 32-bit Microsoft Windows; Sinauer, Associates, Sunderland, Mass.).

A dichotomous cultivar identification key was constructed using the verified AFLP binary data. The cultivars were separated first into two groups based on presence or absence of a peak with primer combination 5.3 (Table 2). The two groups were then subdivided further by identifying another peak that separated each group. This process continued until each cultivar was uniquely identified on the key. Amplified fragment length polymorphism markers from six primer combinations (Table 2) were used to create the key to make it more practical and functional. Peaks or markers that were unique to individual cultivars were included as identity confirmations. Seven anonymous (known only to R.N. Trigiano) samples of *C. florida* cultivars were analyzed to test the validity of the dichotomous cultivar identification key. Unknowns were analyzed with the six selective primer combinations used to construct the dichotomous cultivar identification key.

Results

A total of 204 AFLP fingerprints were produced using 12 primer combinations, and almost all loci were polymorphic. During manual verification of binary data from the electropherograms, several peaks were noted that were unique to individual cultivars (Fig. 1); however, unique markers could not be described for all cultivars included in the study (Table 3). A similarity index (Table 4) was calculated, and a cluster analysis was performed and visualized with a tree of genetic similarity (Fig. 2), which revealed unexpected differences between the duplicate samples. 'Cherokee Daybreak' duplicate samples were 93% similar to each other and 'Cloud 9' samples were 88% similar to each other. Furthermore, the similarity index between each of these duplicate samples and other cultivars is about the same. However, 'Cherokee Chief' and 'Cherokee Sunset' duplicate samples were only 68% and 64% similar respectively and showed large variation between each individual and other cultivars (Table 4). Most nodes on the cladogram were not well supported (<50) by bootstrap values.

A dichotomous key for cultivar identification (Fig. 3) was constructed using the corrected binary data from six primer combinations. The key was tested with seven anonymous *C. florida* samples and six were identified correctly. 'Cherokee Brave' and 'Cherokee Chief' samples were difficult to distinguish using the AFLP markers and the key.

Discussion

Flowering dogwood is a natural obligate outcrossing species and therefore high genetic variation in wild populations is expected (Witte et al., 2000), leading to individuals in natural populations that are highly heterozygous. Many of cultivated lines of dogwoods are selected from these heterozygous wild

Table 2. Amplified fragment length polymorphism primer combinations used to construct the dichotomous cultivar identification key and to identify unique markers of *Cornus florida* cultivars and lines.

Primer code	Primer composition
1.2	E + ACG/M + CAA
1.3	E + ACG/M + CAC
2.3	E + ACA/M + CAC
3.3	E + ACC/M + CAC
4.1	E + ACT/M + CAG
5.3	E + AGA/M + CAC

E, *Eco* RI; M, *Mse* I.

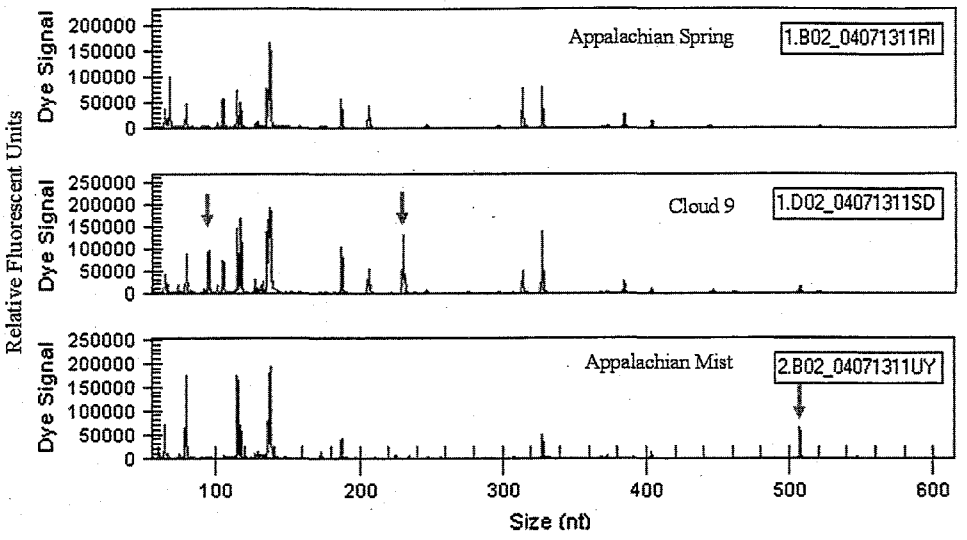

Fig. 1. Amplified fragment length polymorphism fingerprints (electropherograms) generated using the primer combination E + ACG/M + CAA for *Cornus florida* 'Appalachian Spring', 'Cloud Nine', and 'Appalachian Mist'. Note the unique markers (arrows) for 'Cloud Nine' and 'Appalachian Mist'. Size (nt) = size of fragment in nucleotides.

Table 3. Unique amplified fragment length polymorphism markers for *Cornus florida* cultivars and lines.

Cultivar or line	Primer[z]	Unique markers (bp)
Appalachian Blush	1.3	297, 330
Appalachian Mist	5.3	501
	4.1	93, 235
Appalachian Snow	1.3	128, 228
Appalachian Spring	3.3	112
Cherokee Brave	1.3	441
	4.1	275
	3.3	214, 283, 410
Cherokee Chief	4.1	97, 393
Cherokee Daybreak	3.3	196
Cherokee Princess	1.2	230
	5.3	214
Cherokee Sunset	1.3	448
	5.3	138, 169, 202
Cloud 9	2.3	617
Fragrant Cloud	1.3	202
MW 95-4	3.3	70
MW 95-12		None
MW 95-28	4.1	353
	3.3	244
MW 94-60	2.3	101, 380
MW 94-67		None
Plena		None

[z]Primer combination numbers correspond to numbers provided in Table 2.

populations, and thus individual cultivars differ genetically when compared with other cultivars. This is reflected in the similarity index—the average value is about 0.75. 'Cherokee Sunset' and 'Cherokee Brave' were developed from seedlings obtained from 'Cherokee Chief' (unknown male) and were expected to have more similar values (closer to 1.00) to the parent. However, the male parents for these two cultivars were unknown and the calculated genetic distances were similar to other nonrelated individuals within the study.

In many plants, the phenotypic similarity of different cultivars requires an accurate method of identification that is not based on general morphological and horticultural characteristics. DNA fingerprinting methods can be used to distinguish closely related individuals, such as different cultivars, from one another. Although the AFLP fingerprints and unique markers used in this study of *C. florida* were applied to identify correctly most of the unknown samples, there appears to be some genetic variability (about 10%) between individual plants of some cultivars. This variability would be unexpected for clonally propagated cultivars. Assuming that the individuals used as standards were not mislabeled, these results may also indicate some inherent variations in the AFLP technique when used for determining genetic similarity of individual trees. The level of diversity illustrated between some duplicate trees in this study could be considered noise incited by stochastic variation in DNA fragments among the samples. Despite some differences in the overall AFLP profiles for different trees of cultivars, the unique AFLP markers used for identifying cultivars were consistently present in both trees of the same cultivar.

Mislabeling of cultivars is a common occurrence in the nursery industry. For example, 'Rubra', a generic pink-to-red blooming dogwood, could be sold as either 'Cherokee Chief' or 'Cherokee Brave' or some other red-bracted flowering dogwood. Furthermore, the cultivars Barton and Cloud 9 were revealed to be the same cultivar using molecular markers, although they had been sold as two distinctly different cultivars for many years (Windham and Trigiano, 1998). In this case, the two cultivars have similar-appearing inflorescences and could be easily confused. Another example involves *C. kousa* Hance cultivars. 'Rosabella' has been sold as a red-bracted cultivar, but has also been sold as a different cultivar, Miss Satomi (Dirr, 1998); and *C. kousa* 'Heart Throb' was shown to have significant genetic similarity to both 'Rosabella' and 'Miss Satomi'. These findings suggested that either 'Rosabella' may have been sold under three different names or, if the three cultivars are different, they may have been founded from very closely related seedlings, which were selected to have very similar phenotypic characteristics (Trigiano et al., 2004).

A possible explanation of the apparent variation among the different samples of 'Cherokee Sunset' and 'Cherokee Chief' in this study is mislabeling of the original samples (Trigiano et al., 2004; Windham and Trigiano, 1998). In most cases, the trees used in the study were juvenile (not blooming) and leaves from many of these cultivars are virtually indistinguishable. The similarity data indicate that in both cases, two very different genotypes were included as samples of the same cultivar. Mislabeling is not only a problem in the nursery industry, but may have created a problem in this project, because our cultivar standards used for AFLP analyses were used to identify other unknown samples. However, this does not explain the apparent

Table 4. Similarity matrix based on Jaccard coefficient for *Cornus florida* cultivars and lines.[z]

	AB	AM	ASN	AS	CB	CCA	CCB	CDA	CDB	CP	CSA	CSB	C9A	C9B	FC	MWA	MWB	MWC	MWD	MWE	PL
AB	1.00																				
AM	0.63	1.00																			
ASN	0.70	0.70	1.00																		
AS	0.71	0.72	0.83	1.00																	
CB	0.66	0.71	0.78	0.80	1.00																
CCA	0.76	0.72	0.81	0.84	0.88	1.00															
CCB	0.54	0.57	0.48	0.63	0.73	0.68	1.00														
CDA	0.77	0.68	0.86	0.84	0.84	0.86	0.48	1.00													
CDB	0.89	0.72	0.82	0.82	0.79	0.88	0.83	0.93	1.00												
CP	0.61	0.69	0.75	0.80	0.76	0.80	0.73	0.68	0.72	1.00											
CSA	0.50	0.52	0.63	0.66	0.79	0.69	0.83	0.84	0.74	0.70	1.00										
CSB	0.80	0.72	0.82	0.84	0.77	0.87	0.85	0.80	0.83	0.79	0.64	1.00									
C9A	0.77	0.69	0.88	0.87	0.81	0.90	0.86	0.90	0.87	0.80	0.73	0.82	1.00								
C9B	0.69	0.67	0.82	0.80	0.85	0.81	0.86	0.89	0.84	0.79	0.78	0.78	0.88	1.00							
FC	0.74	0.71	0.82	0.90	0.83	0.87	0.69	0.84	0.85	0.80	0.73	0.85	0.86	0.79	1.00						
MWA	0.83	0.69	0.82	0.90	0.83	0.87	0.77	0.87	0.87	0.86	0.70	0.83	0.87	0.87	0.86	1.00					
MWB	0.76	0.71	0.82	0.83	0.77	0.84	0.64	0.87	0.85	0.77	0.68	0.81	0.85	0.79	0.86	0.87	1.00				
MWC	0.83	0.69	0.84	0.85	0.71	0.83	0.59	0.77	0.80	0.74	0.52	0.80	0.74	0.83	0.80	0.82	0.80	1.00			
MWD	0.72	0.73	0.84	0.87	0.82	0.85	0.65	0.82	0.81	0.81	0.84	0.81	0.84	0.84	0.83	0.87	0.82	0.79	1.00		
MWE	0.83	0.76	0.80	0.80	0.75	0.81	0.68	0.79	0.82	0.77	0.57	0.85	0.81	0.80	0.82	0.81	0.82	0.78	0.75	1.00	
PL	0.81	0.68	0.79	0.84	0.84	0.87	0.64	0.82	0.83	0.77	0.70	0.82	0.85	0.79	0.83	0.83	0.82	0.81	0.86	0.77	1.00

[z]AB, 'Appalachian Blush'; AM, 'Appalachian Mist'; AS, 'Appalachian Spring'; ASN, 'Appalachian Snow'; CB, 'Cherokee Brave'; CCA, 'Cherokee Chief' duplicate tree A; CCB, 'Cherokee Chief' duplicate tree B; CDA, 'Cherokee Daybreak' duplicate tree A; CDB, 'Cherokee Daybreak' duplicate tree B; CP, 'Cherokee Princess'; CSA, 'Cherokee Sunset' duplicate tree A; CSB, 'Cherokee Sunset' duplicate tree B; C9A, 'Cloud 9' duplicate tree A; C9B, 'Cloud 9' duplicate tree B; FC, 'Fragrant Cloud'; MWA, MW 95–12, MWB, MW 95-28; MWC, MW 95-4; MWD, MW 94-60; MWE, MW 94-67, and PL, 'Plena'.

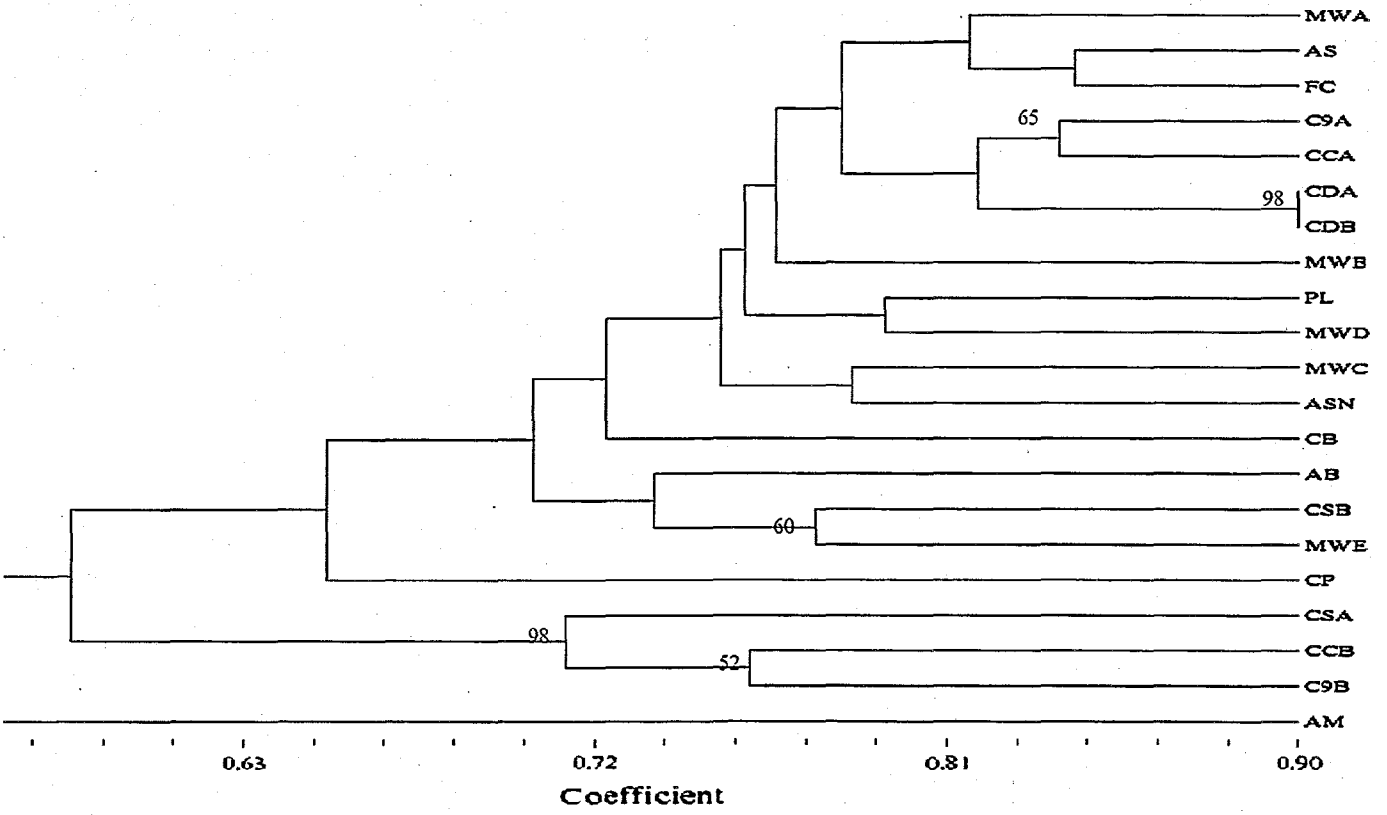

Fig. 2. Cladogram of *Cornus florida* cultivars and lines. Only bootstrap values ≥50 for nodes are included. AB, 'Appalachian Blush'; AM, 'Appalachian Mist'; AS, 'Appalachian Spring'; ASN, 'Appalachian Snow'; CB, 'Cherokee Brave'; CCA, 'Cherokee Chief' duplicate tree A; CCB, 'Cherokee Chief' duplicate tree B; CDA, 'Cherokee Daybreak' duplicate tree A; CDB, 'Cherokee Daybreak' duplicate tree B; CP, 'Cherokee Princess'; CSA, 'Cherokee Sunset' duplicate tree A; CSB, 'Cherokee Sunset' duplicate tree B; C9A, 'Cloud 9' duplicate tree A; C9B, 'Cloud 9' duplicate tree B; FC, 'Fragrant Cloud'; MWA, MW 95-12; MWB, MW 95-28; MWC, MW 95-4; MWD, MW 94-60; MWE, MW 94-67; and PL, 'Plena'.

differences between the two 'Cherokee Sunset' samples, because the leaves of this cultivar are yellow variegated and very distinctive from almost all other cultivars, especially those included in this study. This degree of variation was unexpected because dogwood cultivars are exclusively propagated asexually via bud grafting or rooted cuttings. Genomic DNA was isolated twice from each tree of 'Cherokee Sunset' and 'Cherokee Chief'. Amplified fragment length polymorphisms were generated from the independent DNA isolations and each time failed to provide similar results for the duplicate samples for each of the cultivars. However, AFLP profiles from independent DNA isolations from the same tree of each cultivar were consistent with each other. In contrast, individual trees of 'Cherokee Daybreak', which has distinctive white-variegated leaves, showed little variation. Additional study will be necessary to resolve the disparity between individual trees of 'Cherokee Sunset'.

There is a need for a method of identifying *C. florida* cultivars based on genotype rather than phenotypic characteristics, which are often similar among cultivars. The dichotomous cultivar identification key was constructed using informative AFLP markers that could be used to identify most *C. florida* cultivars within this inclusion group. This cultivar identification key could be useful to breeders, nurserymen, and researchers for patent protection; identification of desirable traits; and identification of unknown cultivars in this inclusion group. When tested with seven anonymous dogwood samples, the key was used to identify six samples successfully. One unknown was unidentifiable using the key—a 'Cherokee Brave' sample. When compared with the 'Cherokee Brave' sample used to create the key, the fingerprint was very different. It is suspected that the original 'Cherokee Brave' sample used to construct the keys may have been mislabeled, because both of the unknown 'Cherokee Brave' samples used to test the key were authentic (from the original block of plants at Commercial Nursery, Inc., Decherd, Tenn.). Additionally, 'Cherokee Brave' and 'Cherokee Chief' samples were difficult to distinguish using only the AFLP markers and the key.

AFLP is a powerful and reproducible technique for many applications in genetics, and has a distinct advantage in recording and computer manipulation of large databases. However, it can have some limitations, especially in identification of very closely related cultivars. For example, a study of poinsettia (*Euphorbia pulcherrima* Willdenow ex Klotzsch) revealed that AFLP markers were unable to distinguish individual series (Parks and Moyer, 2004). In other similar studies, AFLP markers were unable to differentiate cultivars of olive trees (*Olea europea* L.) (Belaj et al., 2004), and pistachio (*Pistacia vera* L.) and pomegranate (*Punica granatum* L.) lines could not be distinguished from each other by AFLP using 16 different primer combinations (R.N. Trigiano, unpublished). Furthermore, a study of azalea (*Rhododendron simsii* Planch) and *Phalaenopsis* concluded that the AFLP technique was limited in the ability to distinguish bud sports from original cultivars (DeRiek et al., 2001). In many plant species, different cultivars are sports of other cultivars, resulting from a single or few gene mutations (Witte et al., 2000). However, 'Cherokee

A. Primer 5.3 at 296/297 bp.....go to B. (MW95-12, 'Cherokee Princess,' 'Cherokee Sunset,' MW95-4, 'Appalachian Blush,' 'Cloud 9,' 'Plena,' MW94-67, 'Cherokee Daybreak,' MW94-60)
A.' Primer 5.3 absent at 296/297 bp .....go to K. ('Appalachian Spring,' Cherokee Chief,' MW95-28, 'Appalachian Mist,' 'Fragrant Cloud,' 'Appalachian Snow,' 'Cherokee Brave')

B. Primer 5.3 at 193 bp..... go to C.
B.' Primer 5.3 absent at 193 bp ..... go to J.

C. Primer 5.3 at 138/139 bp..... **'Cherokee Sunset'** (confirmation Primer 5.3 at 105 bp)
C.' Primer 5.3 absent at 138/139 bp .....go to D.

D. Primer 5.3 at 144 bp..... **MW 95-4** (confirmation Primer 2.3 at 395 bp, 404 bp)
D.' Primer 5.3 absent at 144 bp ..... .go to E.

E. Primer 5.3 at 366 bp .....go to F.
E.' Primer 5.3 absent at 366 bp.....go to G.

F. Primer 5.3 at 232 bp .....go to H.
F.' Primer 5.3 absent at 232 bp..... go to I.

G. Primer 5.3 at 398 bp..... **'Cherokee Princess'** (confirmation Primer 5.3 at 214 bp)
G.' Primer 5.3 absent at 398 bp ..... **'Cloud 9'** (confirmation Primer 2.3 at 617/618 bp)

H. Primer 1.3 at 127 bp..... **'Appalachian Blush'** (confirmation Primer 1.3 at 297 bp, 330 bp)
H.' Primer 1.3 absent at 127 bp ..... **MW 95-12** (confirmation Primer 1.2 at 223 bp)

I. Primer 5.3 at 137 ..... **MW 94-60** (confirmation Primer 1.1 at 68 bp)
I.' Primer 5.3 absent at 137..... **MW 94-67** (confirmation Primer 3.3 at 303 bp)

J. Primer 2.3 at 677 bp **'Plena'**
J.' Primer 2.3 absent at 677 bp ..... **'Cherokee Daybreak'** (confirmation Primer 3.3 at 196 bp)

K. Primer 1.3 at 202 bp..... **'Fragrant Cloud'** (confirmation Primer 1.2 at 302 bp)
K.' Primer 1.3 absent at 202 bp ..... go to L.

L. Primer 1.3 at 228 bp .....**'Appalachian Snow'** (confirmation Primer 3.3 at 128 bp)
L.' Primer 1.3 absent at 228 bp..... ...go to M.

M. Primer 3.3 at 112 bp..... **'Appalachian Spring'**
M.' Primer 3.3 absent at 112 bp .....go to N.

N. Primer 3.3 at 210 bp, 214 bp..... **'Cherokee Brave'** (confirmation Primer 4.1 at 275 bp)
N. Primer 3.3 absent at 210 bp, 214 bp.....go to O.

O. Primer 3.3 at 294 bp..... **'Appalachian Mist'** (confirmation Primer 4.1 at 93, 235 bp)
O.' Primer 3.3 absent at 294 bp .....go to P.

P. Primer 3.3 at 245 bp ..... **'Cherokee Chief'** (confirmation Primer 3.3 at 697 bp)
P.' Primer 3.3 absent at 245 bp..... **MW 95-28** (confirmation Primer 4.1 at 353 bp)

Fig. 3. Dichotomous cultivar identification key for *Cornus florida* cultivars and lines based on amplified fragment length polymorphism markers.

Brave' supposedly originated from a seedling of 'Cherokee Chief' (Cappiello and Shadow, 2005), and the two cultivars are difficult to distinguish with the AFLPs generated in this study. A simple sequence repeat study (X. Wang, R.N. Trigiano, and M.T. Windham, unpublished) of many flowering dogwood cultivars also failed to differentiate these cultivars, but DNA amplification fingerprinting (Trigiano and Caetano-Anollés, 1998), another arbitrarily primed DNA profiling method, identified unique amplification products for both 'Cherokee Chief' and 'Cherokee Brave' (Trigiano et al., 2003).

In conclusion, AFLP markers provide useful information to identify most flowering dogwood accessions. However, care must be taken to obtain authentic samples, and more than one sample of a cultivar or line should be included in the study. For the flowering dogwood cultivars that are not well discriminated by AFLPs, the base information generated by this technique may need to be augmented with data from other fingerprinting techniques, especially for distinguishing individuals from small groups of very closely related cultivars.

Literature Cited

Amador, D.M., D. Brazeau, B. Farmerie, A. Blake, G. Clark, and M. Whitten. 2001. Amplified fragment length polymorphisms (AFLP) workshop. Interdisciplinary Center for Biotechnology Research. University of Florida, Gainesville, Fla.

Belaj, A., L. Rall, I. Trujillo, and L. Baldoni. 2004. Using RAPD and AFLP markers to distinguish individuals obtained by clonal selection of 'Arbequina' and 'Manzamilade Sevilla' olive. HortScience 39:1566–1570.

Cappiello, P. and D. Shadow. 2005. The genus *Cornus*: Dogwoods. Timber Press, Portland, Ore.

DeRiek, J., J. Dendauw, L. Leus, M. DeLoose, and E. VanBockstaele. 2001. Variety protection by use of molecular markers: Some case studies on ornamentals. Plant Biosystematics 135:107–113.

Dirr, M.A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 5th ed. Stipes Publications, Champaign, Ill.

Habera, L.H., K.H. Lamour, R. Donahoo, and N.R. Smith. 2004. A single primer strategy to fluorescently label selective AFLP reactions. Biotechniques 37:902–904.

Parks, E.J. and J.W. Moyer. 2004. Evaluation of AFLP in poinsettia: Polymorphism selection, analysis and cultivar identification. J. Amer. Soc. Hort. Sci. 129:863–869.
Saunders, J.A., S. Mischke, and A.A. Hemeida. 2001. The use of AFLP techniques for DNA fingerprinting in plants. CEQ 2000XL application information. Beckman Coulter, Inc., Fullerton, Calif.
Savelkoul, P.H.M., H.J.M. Aarts, J. deHaas, L. Dijkshoorn, B. Duim, M. Otsen, J.L.W. Rademaker, L. Shouls, and J.A. Lenstra. 1999. Amplified fragment length polymorphism analysis: The state of an art. J. Clin. Microbiol. 37:3083–3091.
Trigiano, R.N., M.H. Ament, and M.T. Windham. 2003. DNA fingerprints nail the clones: How genetic mapping technology is revealing many cultivars' true colors. Nursery Manage. Production 19: 43–46.
Trigiano, R.N., M.H. Ament, M.T. Windham, and J.K. Moulton. 2004. Genetic profiling of red-bracted *Cornus kousa* cultivars indicates significant cultivar synonymy. HortScience 39:489–492.
Trigiano, R.N. and G. Caetano–Anollés. 1998. Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. HortTechnology 8:413–423.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407–4414.
Vos, P. and M. Kuiper. 1997. AFLP analysis, p. 135–153. In: G. Caetano-Anollés and P.M. Gresshoff (eds.). DNA markers. Wiley-Liss, New York.
Weising, K., H. Nybom, K. Wolff, and W. Meyer. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, Fla.
Windham, M.T. and R.N. Trigiano. 1998. Are 'Barton' and 'Cloud 9' the same cultivar of *Cornus florida* L.? J. Environ. Hort. 16:163–166.
Witte, W.T., M.T. Windham, A.S. Windham, F.A. Hale, D.C. Fare, and W.K. Clatterbuck. 2000. PB1670: Dogwoods for American gardens. University of Tennessee Agriculture Extension Service, Knoxville, Tenn.