DNA Amplification Fingerprinting Identifies Closely Related *Chrysanthemum* Cultivars

M.C. Scott, G. Caetano-Anollés¹, and R.N. Trigiano²

Department of Ornamental Horticulture and Landscape Design, Institute of Agriculture, The University of Tennessee, Knoxville, TN 37901-1071

Additional index words. arbitrary primers, cluster analysis, *Dendranthema grandiflora*, DNA bulking, genetic variability, principal coordinate analysis

Abstract. DNA amplification fingerprinting (DAF) was used to study genetic relationships between closely related chrysanthemum cultivars (*Dendranthema grandiflora* Tzvelev.). Twenty-one cultivars were examined that belonged to the Anne, Blush, Boaldi, Charm, Davis, and Pomona series (families). The genetic variability of cultivars within and between series was evaluated using eleven arbitrary octamer primers. A few polymorphic characters uniquely identified closely related cultivars within each of the series. In contrast, many DNA polymorphisms were observed between members of the different series. Phenetic patterns were established by unweighted pair group cluster analysis using arithmetic means (UPGMA) and principal coordinate analysis (PCO). The average distance between series was 10-fold greater than between cultivars within a series. DNA from all cultivars belonging to a series were also bulked to generate profiles containing unique amplified products for each series. Cluster analysis and PCO of bulked DNA clearly grouped Charm and Pomona together. However, series grouping did not correspond to morphology of inflorescence types. The results demonstrate the utility of the DAF technique in distinguishing clonal materials and its potential use for patent protection, phylogenetic studies, and for identifying useful markers in breeding applications.

Floriculture is a billion dollar industry and chrysanthemum [*Dendranthema grandiflora* or *Chrysanthemum morifolium* Ramat (synonym) Anderson (1987)], has been valued at more than \$150 million in the United States alone (U.S. Dept. of Agriculture, 1992). Despite the economic importance of floricultural products, the industry has not generally embraced the use of molecular techniques to support breeding or the legal protection of newly developed cultivars. The genetic characterization of floricultural crops has been applied only recently to relatively few species including roses (Hubbard et al., 1992; Rajapakse et al., 1992; Torres et al., 1993), petunia (*Petunia* species Juss.) (Cerny et al., 1996), and chrysanthemum (Wolff et al., 1993; 1994). Some prospective applications of molecular genetics in floriculture include varietal protection, cultivar certification, marker assisted selection (MAS), linkage mapping, and genetic engineering.

Many new chrysanthemum cultivars have originated via somatic mutation (Dowrick and El-Bayoumi, 1966). Consequently, there is a need to differentiate vegetatively derived accessions. The accurate identification of cultivars is important to the horticulture industry because many are legally protected. In the past it has been difficult to prove patent infringement; a reliable technique that could accurately differentiate between phenotypically similar, though genetically different, plants was not available (Hubbard et al., 1992). The recent advent of DNA fingerprinting has alleviated such limitations.

DNA fingerprinting techniques generally use the hybridization or amplification of nucleic acids to generate patterns characteristic of a particular organism. Restriction fragment-length polymorphism (RFLP) analysis and amplification-based nucleic acid scan-

ning methodologies, such as randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990) and DNA amplification fingerprinting (DAF) (Caetano-Anollés et al., 1991), have been used profusely in genome identification. RFLP analysis (Wolff et al., 1994) and RAPD fingerprinting (Wolff et al., 1995; Wolff and Peters-Van Rijn, 1993) were used recently to study chrysanthemum. RFLP probes and locus-specific PCR primers were developed for future genetic variability and MAS studies (Wolff et al., 1994). However, the RFLP technique was found to be laborious and not well-suited for studies involving large numbers of samples. In contrast, RAPD analysis was fast and relatively simple to perform and as such was used to assess genetic variability of species and closely related cultivars of chrysanthemum (Wolff et al., 1993). However, accessions that were somatic mutants of the original cultivar could not be distinguished from their parents. Therefore, a method sensitive enough to differentiate cultivars within series (also known as families) of chrysanthemum was deemed necessary.

DAF uses very short oligodeoxynucleoside primers, seven or eight nucleotides in length, to produce relatively complex DNA profiles. These arbitrary primers amplify multiple genomic regions (amplicons), many of which are polymorphic and represent allelic differences that can be traced in inheritance studies or used to identify individuals (Caetano-Anollés et al., 1991). Because of the high information content of fingerprints, DAF has been successfully used to identify closely related organisms including bacteria, fungi, plants, and humans (Caetano-Anollés et al., 1991). In this study, DAF was used to assess the genetic variability of groups of closely related cultivars of chrysanthemum that belong to six different series and to develop markers for the unequivocal identification of cultivars and series.

Materials and Methods

Plant material. Rooted cuttings of 21 nonhardy pot-type chrysanthemum cultivars belonging to six series (Table 1) were obtained from Yoder Brothers, Inc., Barberton, Ohio. Each cultivar in a series is a sport or somatic mutant that arose from another

Received for publication 18 Mar. 1996. Accepted for publication 26 July 1996. We thank Yoder Brothers, Inc., for donating the plant material and The Tennessee Agricultural Experiment Station for providing financial support. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Plant Molecular Genetics, Institute of Agriculture and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901-1071.

²To whom reprint requests should be addressed.

Table 1. Chrysanthemum series and cultivars.

Series	Inflorescence type	Cultivar
Anne	Decorative	Bright Golden Anne Cream Yellow Princess Anne Peacock
Blush	Daisy	Blush Coral Blush White Blush
Boaldi	Decorative	Boaldi Yellow Boaldi
Charm	Decorative	Charm Coral Charm Dark Bronze Charm Dark Charm Salmon Charm
Davis	Daisy	Davis Coral Davis Light Davis Regal Davis
Pomona	Decorative	Pomona Cherry Pomona Coral Pomona Dark Pomona

cultivar within that series and phenotypically differs primarily only in flower color. Cultivars in the Anne, Boaldi, Charm, and Pomona series have a decorative flower type, whereas cultivars in the Davis and Blush series have a daisy-type flower. Nine plants of each cultivar were grown in the laboratory to prevent insect and disease infestation and to limit carbohydrate accumulation that had been previously demonstrated to interfere with DNA isolation (Trigiano, unpublished data). Plants were maintained vegetatively under fluorescent and incandescent lights providing about 75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 16 h a day. Plants were pinched twice a month, watered once a week, and fertilized once a week with 300 ppm Peters 20–10–20. Terminal cuttings were made and rooted twice a year to maintain vigorously growing plants.

DNA extraction and amplification. DNA was extracted from about 50 mg of young, not yet fully expanded, light-green leaves using the method of Yoon et al. (1991) as modified by Trigiano et al. (1995). Leaves were either used fresh or frozen ($-70\text{ }^{\circ}\text{C}$). Additionally, 100 mg of polyvinyl-polypyrrolidone was used in the extraction buffer to sequester phenolic compounds. DNA was extracted at least three times for each cultivar in each series, except for the cultivars in the Blush and Boaldi series, which were bulked. DNA was quantified by fluorescent enhancement of the dye H33258 and using a fluorometer (TKO 100; Hoefer, San Francisco). The extraction procedure consistently yielded between 1.75 and 8.75 μg of DNA/50 mg of tissue.

DNA stocks were diluted with sterile distilled water to final concentrations of 0.5 $\text{ng}\cdot\mu\text{L}^{-1}$ and amplified according to the method of Caetano-Anollés et al. (1991) with the following modifications.

The reaction mixture (10 μL) contained 4 units of Stoffel fragment Amplitaq DNA polymerase, 1 μL of TTK10 buffer (20 mM Tris-HCl; 0.1% Triton X-100; 4 mM $(\text{NH}_4)_2\text{SO}_4$; 10 mM KCl), 1 ng of template DNA, 200 μM of dNTP, 2.5 mM MgSO_4 , and 3 μM of primer. Primers had the following (5'–3') sequences: GAGCCTGT, GTAACGCC, GATGCAGG, GAAACGCC, GTTACGCC, GTATCGCC, GTAACCGC, AATGCAGC, CCGAGCTG, CGTGGTGG, and CGCGGCCA. Reactions were

amplified in a thermocycler (Easy Cycler Twin Block System; Ericomp Inc., San Diego, Calif.) for 35 cycles (each lasting about 5.5 min) consisting of 10-s steps at 30 and 96 $^{\circ}\text{C}$. Preliminary experiments showed that an extension step at 72 $^{\circ}\text{C}$ was unnecessary to produce complex and reproducible profiles. Amplifications were repeated at least three times for each primer–template combination and demonstrated that, although some variation in staining intensity was evident, overall profile patterns were consistent from independent DNA isolations and amplifications (data not shown). For example, identical profiles were obtained by amplification of DNA isolated from three individual plants of 'Salmon Charm' (Fig. 1A).

In the analysis of bulked samples, equal amounts of DNA from each cultivar within a series were mixed together and then amplified. Alternatively, equal weights of leaves from each cultivar were blended together, and the DNA was extracted and then amplified. Bulking DNA from all members of a series provided the opportunity to identify unique amplification products characteristic of that series. Amplification products present in individual fingerprints were represented in fingerprints of the bulks.

Amplification products, sometimes diluted 1:1 with sterile distilled water, were separated electrophoretically on 4.5% polyacrylamide gels backed on polyester film (GelBond PAG; FMC, Rockland, Maine) as previously described (Trigiano et al., 1995). DNA was visualized using a fast and sensitive silver staining procedure that detects picogram quantities of DNA (Bassam et al., 1991).

Data analyses. Silver stained gels were examined on a light box and bands of 700 base pairs (bp) or less scored as either present (1) or absent (0). Products of higher molecular weight were not considered. At least two gels for each primer–cultivar combination were compared to ensure that the profiles were consistent. In a few cases, questionable products were assigned as missing data. Phenetic analyses were performed using numerical taxonomy and multivariate analysis system (NTSYS-pc) version 1.7 (Exeter Software, Setauket, N.Y.). In principal coordinate analyses (PCO),

Fig. 1. DNA amplification profiles of individual plants and cultivars of a series. (a) Profiles generated by the primer GAGCCTGT (5'–3') for three individual plants of 'Salmon Charm' affirming clonal fidelity. Polymorphisms were not detected between individual plants. (b) DNA amplification profiles generated by the primer (5'–3') GATGCAGG for five cultivars of the Charm series and two outgroup members. Polymorphisms between series members are indicated by arrowheads; see Table 2 for molecular weights. Lane 1 = 'Charm', 2 = 'Coral Charm', 3 = 'Dark Bronze Charm', 4 = 'Dark Charm', 5 = 'Salmon Charm', 6 = 'Davis', 7 = 'Pomona'. M = molecular markers in base pairs $\times 1000$.

Dice similarity coefficients were calculated and squared genetic distances aligned using the double center option. Eigenvectors were then calculated from the transformed matrix using the square root (lambda) scaling option. The results were graphically displayed without normalization of scales. Data were also analyzed using unweighed pair group cluster analysis using arithmetic means (UPGMA). Genetic similarity matrices for each series and bulks were computed and cluster analyses completed with options set for a maximum of 25 tied trees.

Results

To determine clonal fidelity within a particular cultivar three individual plants of 'Salmon Charm', 'Light Davis', and 'Pomona' were selected and analyzed by DAF with 11 octamer primers. Polymorphic DNA between plants of the same cultivar were not detected (Fig. 1A). These results support the decision to represent each individual cultivar within a specific series by a few sample plants without the need to bulk DNA from many individuals, as was necessary for the heterogenic *Petunia ×hybrida* cultivars (Cerny et al., 1996).

Genomic DNA from 21 cultivars belonging to the series Anne, Blush, Boaldi, Charm, Davis, and Pomona were amplified with the set of 11 primers. The number of bands produced for each primer/template combination varied from 13 to 41 with a mean of 28 scored at or below 700 bp. More than 250 bands were analyzed for each chrysanthemum series. Despite variability in staining intensity and cross-sectional areas of some bands, the overall profile patterns remained reproducible between independent DNA isola-

tions and amplifications for all primer-template combinations (Fig. 1A). Cultivars were clearly distinguished within most series. DNA polymorphisms were detected by six primers in one or more series, whereas, five primers rendered monomorphic patterns. There were 13 DNA polymorphisms detected within the Anne series, 6 within Charm, 12 within Davis, and 16 within Pomona (Table 2).

Several octamer primers detected polymorphic DNA in cultivars of the Anne series (Table 2). Of 251, 13 (5.2%) character loci were polymorphic and of sizes ranging from 100 to 300 bp. 'Bright Golden Anne' and 'Cream Yellow Princess Anne' were uniquely identified by amplification products generated with the primer GTAACGCC (100 and 175 bp, respectively), whereas 'Peacock' was distinguished from the other cultivars by two amplification products produced by GATGCAGG (160 and 115 bp).

Polymorphisms in members of the Charm series were shown by the primers GATGCAGG and GTATCGCC. Only 6 of 270 (2.2%) character loci were polymorphic and ranged between 100 and 300 bp (Table 2, Fig. 1B). In this series, identification of specific cultivars was not clear-cut. For example, 'Dark Bronze Charm' and 'Dark Charm' were differentiated within the series by three bands produced by GATGCAGG (200, 180, and 130 bp). 'Salmon Charm' was identified by a band generated by GTATCGCC (250 bp) and by a band produced by GATGCAGG (200 bp), which differentiated it from 'Dark Bronze Charm'. 'Charm' and 'Coral Charm' were not clearly distinguished from the other members of the series.

Within the Davis series, 12 of 278 (4.3%) character loci were polymorphic. These bands were between 100 and 320 bp and

Table 2. DNA amplification polymorphisms (AFLPs) generated by six octamer primers within four series of chrysanthemum.

Primer	Series ^z																			
	Anne				Charm						Davis					Pomona				
	1	2	3	AFLP (bp)	1	2	3	4	5	AFLP (bp)	1	2	3	4	AFLP (bp)	1	2	3	4	AFLP (bp)
GAGCCTGT	1 ^y	1	0	250, 160						---	0	0	1	0	160	0	1	1	1	205
																0	0	0	1	200
																0	1	1	0	140
																1	1	0	0	135
																0	1	0	1	130
GTAACGCC	1	0	1	180						---	1	1	0	1	195	1	1	1	0	270
	0	1	0	175																
	1	0	0	100																
GATGCAGG	1	1	0	240	0	1	1	0	0	270, 145	1	0	1	0	210	0	1	0	0	310
	0	0	1	160, 115	0	0	1	0	0	200, 180	1	0	0	0	200	1	0	0	0	210, 190, 140
	0	0	0	1	0	130														
GTTACGCC	1	0	0	260						---					---	1	0	0	0	310
																1	1	0	1	270, 210
																1	1	0	0	200
GTATCGCC	0	1	1	290, 140	0	0	1	0	1	250	1	0	0	1	315	0	1	0	0	225
	0	1	0	200, 155							1	1	0	0	250	0	1	0	1	200
											1	0	1	1	245, 215					
											0	1	0	0	240					
											1	1	0	1	210, 130					
AATGCAGC				---						---	0	1	0	0	140					---

²Cultivars in each series are identified by numbers, outgroup data are not shown. Anne series: 1 = 'Bright Golden Anne', 2 = 'Cream Yellow Anne', 3 = 'Peacock' (outgroup = 'Charm' and 'Pomona'); Charm series: 1 = 'Charm', 2 = 'Coral Charm', 3 = 'Dark Bronze Charm', 4 = 'Dark Charm', 5 = 'Salmon Charm' (outgroup = 'Davis' and 'Pomona'); Davis series: 1 = 'Coral Davis', 2 = 'Davis', 3 = 'Light Davis', 4 = 'Regal Davis' (outgroup = 'Charm' and 'Pomona'); Pomona series: 1 = 'Cherry Pomona', 2 = 'Coral Pomona', 3 = 'Dark Pomona', 4 = 'Pomona' (outgroup = 'Charm' and 'Davis').

^yAmplified product either present (1) or absent (0).

^xDNA polymorphisms not detected.

Table 3. Similarity matrix based on Dice coefficients among five chrysanthemum cultivars in the Charm series and outgroup cultivars 'Davis' and 'Pomona'.

Cultivar	1	2	3	4	5	6	7
Charm (1)	1.000						
Coral Charm (2)	0.993	1.000					
Dark Bronze Charm (3)	0.985	0.991	1.000				
Dark Charm (4)	0.996	0.993	0.985	1.000			
Salmon Charm (5)	0.996	0.993	0.989	0.996	1.000		
Davis (6)	0.884	0.886	0.891	0.888	0.884	1.000	
Pomona (7)	0.926	0.933	0.934	0.931	0.926	0.886	1.000

produced by five primers (Table 2). All cultivars could be identified using one or more primers. 'Coral Davis', 'Davis', and 'Light Davis' were uniquely distinguished by the primers GATGCAGG (200 bp), GTATCGCC (240 bp), and AATGCAGC (140 bp), and GAGCCTGT (160 bp), respectively. Two primers were needed to unequivocally characterize 'Regal Davis'. A band was generated for 'Regal Davis' and 'Coral Davis' using GTATCGCC (315 bp), but the two cultivars could be separated by using a locus specific for 'Coral Davis' produced by GATGCAGG (200 bp).

Polymorphic DNA was detected in DAF profiles of the Pomona series by the primers GAGCCTGT, GTAACGCC, GATGCAGG, GTTACGCC, and GTATCGCC. Of the 283 character loci scored, 16 (5.7%) were polymorphic and ranged between 100 and 320 bp (Table 2). All members of the series could be easily identified by one or more bands. 'Pomona' was identified by a unique band produced by GAGCCTGT (200 bp) and 'Cherry Pomona' was clearly distinguished from other cultivars by products generated with primers GATGCAGG (210, 190, and 140 bp) and GTTACGCC (310 bp). 'Dark Pomona' and 'Coral Pomona' were identified by bands generated with the primers GATGCAGG (310 bp) and GTATCGCC (225 bp), respectively.

Genetic relationships between cultivars within each series were determined by UPGMA and an ordination technique (PCO). Genetic distances between cultivars were calculated within each series and relative to representative cultivars of two other series that were defined as an outgroup. The distances between cultivars within individual series ranged from 0.4% to 3.5% (e.g., Table 3; other analyses not shown) and indicate that their genomes are highly conserved. Conversely, genetic distances between outgroup and individual members of a series ranged from 6.6% to 12.3% (e.g., Table 3; other analyses not shown). UPGMA and PCO were

able to delineate the typically tightly clustered individual cultivars within each series (e.g., Fig. 2).

Analysis of bulked DNA using the 11 octamer primers yielded 113 polymorphic fragments between series, representing 34% of all amplified character loci. Some primers, such as CCGAGCTG, detected polymorphic DNA between series, but could not discriminate cultivars within series. In contrast, primers GATGCAGG and GTATCGCC characterized cultivars both within and between series (Fig. 3).

Amplified profiles generated by similar primers were consistent regardless of the method used to prepare bulk DNA. Genetic distances calculated from bulked DNA of a series compared favorably to the distances between individual cultivars and the outgroup; distances ranged from 9% to 11.2% (Table 4). PCO (Fig. 4A) and cluster analysis (Fig. 4B) clearly differentiated the six series. In UPGMA and PCO, 'Charm' and 'Pomona' were clearly grouped together. However, grouping of the series did not correspond to flower morphology; 'Blush' and 'Davis' have daisy-type inflorescences and clustered relatively close together in PCO, but were more distant in UPGMA. Similarly, no correlation was found with flower color.

Discussion

Members of chrysanthemum series or families originate from each other via somatic mutations, although it is unknown if phenotypic differences result from point mutations, inversions, deletions, or, in the extreme, loss of chromosomes (Wolff and Peters Van Rijn, 1993). Series members often differ only in a

single phenotypic trait such as flower color, and this may only entail subtle changes in hue. Furthermore, most cultivars are derived vegetatively since breeding is exceedingly difficult (Dowick and El-Bayoumi, 1966). Consequently, there is a need for methodologies sensitive enough to detect small differences between closely related cultivars.

The ability to determine cultivar identity within series would be of great value to chrysanthemum breeders

Fig. 2. Graphic representation of principal coordinate analysis (PCO) of the relationship between cultivars in the Charm series and members of an outgroup. Cultivars in the series were cluster tightly together, but distinguishable. Similar representations were obtained for the Anne, Davis, and Pomona series. 1 = 'Charm', 2 = 'Coral Charm', 3 = 'Dark Bronze Charm', 4 = 'Dark Charm', 5 = 'Salmon Charm'; E = 'Davis' and F = 'Pomona'.

Fig. 3. DNA amplification profiles generated by the primer GATGCAGG (5'-3') from bulked DNA of six series of chrysanthemum. Some polymorphisms are denoted by arrow heads. Lane 1 = Anne, 2 = Blush, 3 = Boaldi, 4 = Charm, 5 = Davis, 6 = Pomona; Numbers = molecular markers in base pairs $\times$ 1000.

Table 4. Similarity matrix based on Dice coefficients for bulk analysis of six series of chrysanthemum.

Series	1	2	3	4	5	6
Anne (1)	1.000					
Blush (2)	0.913	1.000				
Boaldi (3)	0.910	0.892	1.000			
Charm (4)	0.901	0.889	0.894	1.000		
Davis (5)	0.888	0.910	0.904	0.897	1.000	
Pomona (6)	0.898	0.879	0.891	0.928	0.895	1.000

and commercial producers. DAF is sufficiently discriminating to detect heterogeneous regions within these relatively homogeneous genomes. For example, the primer GATGCAGG targeted heterogeneous DNA regions that could be used to recognize some cultivars within the Anne, Charm, Davis, and Pomona series. If primers are selected to yield highly polymorphic profiles, then only relatively few primers are necessary to distinguish all closely related cultivars within a series. In contrast, RAPD analysis of other chrysanthemum series was unable to distinguish individual cultivars, which were also derived vegetatively from each other (Wolff and Peters-Van Rijn, 1993). In this study, only 1 of the 27 decamer primers evaluated yielded slightly different RAPD profiles for 13 mutant cultivars contained within a series, but none yielded distinguishing polymorphisms within the series. More recently, another study using RAPD, RFLP, and inter-simple sequence repeat (SSR) PCR analyses, a technique that generates profiles with complexities similar to DAF, could not distinguish closely related accessions of chrysanthemum (Wolff et al., 1995). In contrast, DAF with 6 of 11 primers was able to detect polymorphic DNA in our study. Many of the polymorphisms could be used to unequivocally identify individual cultivars within each examined series. The exception was the Charm series, in which not all cultivars were delineated. However, resolution of these cultivars may be possible by using arbitrary signature of amplified profiles (ASAP) (Caetano-Anollés and Gresshoff, 1996), a novel technique capable of revealing the fine structure of amplified regions in greater detail. This technique genetically characterized regional subpopulations from a relatively homogeneous population of *Discula destructiva* Redlin, a fungus that infects flowering dogwood (Caetano-Anollés, unpublished data).

Another objective was to measure genetic variation within and between series of chrysanthemum cultivars. Estimates of genetic distances or similarities have greater confidence when data sets are sufficiently large and include at least as many informative characters as there are taxa in the study (Stewart, 1993). The distribution of loci over the genome becomes relatively uniform with large data sets, and a relatively accurate assessment of differences can be achieved (Skroch and Nienhuis, 1995; Skroch et al., 1992). Using DAF, more than 250 bands were produced for analysis of each series and provided a sufficient number of informative characters to evaluate relationships between cultivars of the same series. Analysis confirmed the expected, but exceptional, homogeneity between members of the same series (Wolff et al., 1995). In contrast, a RAPD study of closely related cultivars of chrysanthemum utilized more than 2 times the number of decamer primers to produce a lower number of bands, but could not distinguish individual cultivars (Wolff and Peters-Van Rijn, 1993). Similarly, inter-SSR, PCR was unable to differentiate closely related cultivars of chrysanthemum (Wolff et al., 1995). This strongly indicates that DAF is much superior to RAPD and even inter-SSR PCR analysis in the ability to detect polymorphic DNA between closely related organisms.

There are many phenotypical differences between series of cultivars, including, but not limited to, inflorescence type, leaf shape, and flower color, which have been used as taxonomic delineators (Cumming, 1964; Dowrick and El-Bayoumi, 1965). Correspondingly, with greater divergence in morphology, the expected number of polymorphisms and, thus, genetic distances were greater in outgroup and bulk analyses. Inflorescence type is a primary morphological taxonomic trait used to characterize series of chrysanthemum. Groupings of series were only faithful to inflorescence type if data were analyzed by PCO of bulked DNA. Since the six series were only separated by <0.04 distance units and bulking of DNA analysis had the effect of decreasing measured variability between groups (series), cluster analysis did not find a correlation between molecular and morphological characters. This lack of correlation suggests that the genes controlling inflorescence type are not randomly distributed throughout the genome, and therefore, the unrestricted targeting with arbitrary DAF markers appears unlinked to this morphological character. This may also hold true for flower color between series. In contrast, a strong correlation between DAF markers and flower color in petunia has shown an apparently unrestricted distribution of corolla color controlling loci (Cerny et al., 1996).

This study demonstrated that DAF is well-suited for identifying closely related cultivars within series of chrysanthemum. Diagnostic amplification products for most cultivars within a series can be produced reliably and efficiently using relatively few primers. Additionally, by amplifying bulked DNA, distinctive profiles and unique products may be generated that serve to characterize series containing many cultivars. The ability to unambiguously distinguish individuals will be paramount in cultivar identification, marker assisted breeding and genetic mapping applications.

Fig. 4. Graphic representations of phenetic analyses of bulked DNA from six chrysanthemum series. (a) Principal coordinate analysis (PCO) and (b) cluster analysis (CA) using UPGMA.

Literature Cited

- Anderson N.O. 1987. Reclassification of the genus *Chrysanthemum* L. HortScience 22:313.
- Bassam B.J., G. Caetano-Anollés, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA polyacrylamide gels. Anal. Biochem. 196:80–83.
- Caetano-Anollés G. and P.M. Gresshoff. 1996. Generation of sequence signatures from DNA amplification fingerprints with mini-hairpin and microsatellite primers. Biotechniques 20:1044–1056.
- Caetano-Anollés G., B.J. Bassam, and P.M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary primers. Bio/Technology 9:553–557.
- Cerny, T., G. Caetano-Anollés, R.N. Trigiano, and T.W. Starman. 1996. Molecular phylogeny and DNA amplification fingerprinting of *Petunia*. Theor. Appl. Genet. 92:1009–1016.
- Cumming, R.W. 1964. The chrysanthemum book. D. Van Nostrand Co., Princeton, N.J.
- Dowrick G.J. and A. El-Bayoumi. 1966. The origin of new forms of the garden chrysanthemum. Euphytica 15:32–38.
- Dowrick G.J. and A. El-Bayoumi. 1965. The induction of mutations in chrysanthemum using X- and gamma radiation. Euphytica 14:204–211.
- Hubbard, M., J. Kelly, S. Rajapakse, A. Abbott, and R. Ballard. 1992. Restriction fragment length polymorphisms in rose and their use for cultivar identification. HortScience 27:172–173.
- Rajapakse, S., M. Hubbard, J.W. Kelly, A.G. Abbott, and R.E. Ballard. 1992. Identification of rose cultivars by restriction fragment length polymorphism. Sci. Hort. 52:237–245.
- Skroch, P. and J. Nienhuis. 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Genet. 91:1086–1091.
- Skroch, P., J. Tivang, and J. Nienhuis. 1992. Analysis of genetic relationships using RAPD marker data, p. 26–30. In: Applications of RAPD technology to plant breeding. Crop Sci. Soc. Amer., Madison Wisc.
- Stewart, C.-B. 1993. The powers and pitfalls of parsimony. Nature 361:603–607.
- Torres A.M., T. Millan, and J.J. Cubbero. 1993. Identifying rose cultivars using random amplified polymorphic DNA markers. HortScience 28:333–334.
- Trigiano R.N., G. Caetano-Anollés, B.J. Bassam, and M.T. Windham. 1995. DNA amplification fingerprinting provides evidence that *Discula destructiva*, the cause of dogwood anthracnose in North America, is an introduced pathogen. Mycologia 87:490–500.
- U.S. Department of Agriculture. 1992. Agriculture statistics board. Floriculture crops. p. 6, 41, 63.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalksi, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531–6535.
- Wolff, K., E. Zietkiewicz, and H. Hofstra. 1995. Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. Theor. Appl. Genet. 91:439–447.
- Wolff K., J. Peters-Van Rijn, and H. Hofstra. 1994. RFLP analysis in chrysanthemum. I. Probe and primer development. Theor. Appl. Genet. 88:473–478.
- Wolff K. and J. Peters-Van Rijn. 1993. Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev.) using random primers. Heredity 71:335–341.
- Yoon, C.-K., D.A. Glave, and P.D. Shaw. 1991. A method for rapid small-scale preparation of fungal DNA. Mycologia 83:835–838.