Theor Appl Genet (2003) 106:1509–1516
DOI 10.1007/s00122-003-1210-7

Jason Carr · Mingliang Xu · John W. Dudley · Schuyler S. Korban

AFLP analysis of genetic variability in New Guinea impatiens

Received: 13 June 2002 / Accepted: 1 November 2002 / Published online: 13 March 2003

Abstract New Guinea impatiens (*Impatiens hawkeri*) is an economically important floral crop, however, little work has been conducted to further our understanding of the genetics of this crop. In this study, we used amplified fragment length polymorphism (AFLP) technology to investigate the level of polymorphism present among 41 commercial cultivars of New Guinea impatiens, study their genetic relatedness, and assess the genetic diversity in this material. An efficient DNA extraction protocol was developed, and a total of 48 *Eco*RI and *Mse*I primer combinations were used for PCR amplification. Amplification products were then subjected to polyacrylamide gel electrophoresis. The AFLP analysis showed that all 41 cultivars generated between 73 and 130 scoreable polymorphic bands per primer combination. Gower's Genetic Dissimilarity estimates for the entire set of cultivars ranged between 0.940 and 0.488. A dendogram was generated from these dissimilarity data that revealed four groupings among these 41 cultivars. The implications of these results on genotypic variation, genetic relationships, and genetic diversity in New Guinea impatiens will be discussed.

Keywords AFLP markers · Genetic analysis · Germplasm evaluation · *Impatiens hawkeri*

Communicated by P. Langridge

J. Carr · M. Xu · S. S. Korban (✉)
Department of Natural Resources and Environmental Sciences, University of Illinois, 310 Madigan Building, 1201 West Gregory Drive, Urbana, IL 61801, USA,
e-mail: korban@uiuc.edu

J. W. Dudley
Department of Crop Sciences, University of Illinois, 310 Madigan Building, 1201 West Gregory Drive, Urbana, IL 61801, USA,

Introduction

Determining the level of genetic variability present among individuals in a breeding program is essential for the improvement of a crop (Lee 1995). This knowledge also provides guidance in developing an effective breeding scheme for a crop (Mumm and Dudley 1994).

New Guinea impatiens (*Impatiens hawkeri*) is an economically important horticultural crop, but little work has been conducted since the 1970s to further our understanding of the genetics of this crop. The germplasm pool, from which New Guinea impatiens cultivars are derived, encompasses several species previously introduced from New Zealand into the United States in 1970. These introductions, 25 specimens in all, were composed of several different closely related species (Martin 1984). These species included *Impatiens schlecteri* Warb., *Impatiens mooreana* Schlect., *Impatiens linearifolia* Warb. and *Impatiens herzogii* K. Schum. (Arisumi 1982). Crosses among various species of New Guineas often resulted in male-sterile progeny with misshapen and abnormal pollen grains (Arisumi 1973). Cytological studies attributed this male-sterility problem to differences in ploidy levels and chromosome numbers among the various species. The occurrence of different ploidy levels in gametes often resulted in the recovery of progeny with irregular chromosome arrangements (Arisumi 1973). Male sterility has been a major impediment to breeding efforts for improvement of this crop.

Historically, genetic relationships among plants have been estimated based on morphological characteristics, sexual compatibility and taxonomic classification. More recently, molecular markers such as restriction fragment length polymorphisms (RFLPs) have become useful tools for characterizing genetic relationships. Polymerase chain reaction (PCR)-based marker systems, such as randomly amplified polymorphic DNA (RAPD) markers, simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs), have further facilitated studies on genetic variability, genetic relatedness and genetic diversity (Powell et al. 1996). Molecular marker systems

hold distinct advantages over phenotypic-based characterizations, as DNA-based markers are not affected by environmental factors. DNA-based markers also provide more information than can be attained through any other method since unlimited numbers of DNA fragments can be analyzed with additional primer combinations. Diversity estimates based on DNA markers better reflect actual DNA differences than pedigree information as selection pressure and genetic drift are both accounted for (Barrett et al. 1998). Furthermore, levels of detection with DNA-based systems are much higher than previously available with morphological markers (Bretting and Widrelacher 1995).

Studies comparing AFLPs to other molecular marker systems for measurements of genetic diversity among plant species have found the AFLP technique to be as informative as other marker systems, and in many cases more valuable due to a higher level of overall polymorphism (Lu et al. 1996; Powell et al. 1996; Yee et al. 1999; Garcia-Mas et al. 2000; Renganayaki et al. 2001). Other advantages of the AFLP technique include high reproducibility, rapid generation of markers, and observation of large numbers of markers that are randomly distributed throughout the genome (Lin et al. 1996; Breyne et al. 1997).

Similar studies in crops, such as daylily (*Hemerocallis* spp.) (Tomkins et al. 2001), wheat (*Triticum aestivum* L.) (Barrett and Kidwell 1998), soybean (*Glycine max*) (Lin et al. 1996), *Arabidopsis* (Breyne et al. 1999) and rice (*Oryza* spp.) (Aggarwal et al. 1999), found the AFLP technique to be a useful and rapid method for determination of genetic diversity within or among species. Studies showed that AFLP or other molecular marker data generally agreed with available pedigree information (J. Dudley, personal communication). In a recent study, Virk et al. (2000) reported that unmapped AFLP markers were highly reliable in measuring genetic diversity. However, others have reported poor to moderate correlation between genetic similarity values based on AFLP markers and pedigree-based estimates (Schut et al. 1997; Degani et al. 2001).

The objective of this study was to determine the genetic variability and genetic relationships within a group of commercially propagated New Guinea impatiens cultivars. The information obtained through the use of AFLP markers was then compared to available pedigree information in order to assess the validity of the molecular marker data.

Materials and methods

Plant material

The New Guinea impatiens germplasm has been obtained from Ball FloraPlant (Arroyo Grande, Calif). A total of 41 cultivars of New Guinea impatiens belonging to the 'Celebrette' and 'Celebration' series are included in this study (Table 1). The cultivars in these two series are variable in phenotype, and possibly in their genetic background, as they are derived from an original group of 25 plant introductions having desirable horticultural characteristics, first obtained from New Zealand (Martin 1984). As the original plant introductions have been asexually propagated, selections and named cultivars released are likely to be highly heterozygous at various loci. It is important to point out that the pedigrees for most of these cultivars are unknown (Table 1).

Cuttings of all 41 cultivars were placed into 15-cm-diameter plastic pots containing a high porosity soil-less medium (Sun Gro Horticulture, Inc., Bellevue, Wash.). Plants were kept in a greenhouse without supplemental lighting, and received a standard regime of water and fertilization (Ball 1991). Fertilizer was applied weekly, alternating between two types. For 1 week, Excel Cal-Mag (15-5-15; N-P-K) (Scott's-Sierra Horticultural Products Company, Marysville, Ohio) was applied at a concentration of 300 ppm; while, in the following week, the fertilizer Miracid (15–5–15; N–P–K) (Scott's, Marysville, Ohio) was applied at the same concentration.

DNA isolation

Several DNA extraction protocols were attempted using leaf tissue of New Guinea impatiens; however, a high polysaccharide content in the leaf tissue hindered efforts to obtain high quality DNA. Eventually, the best quality DNA was obtained by utilizing the Nucleon Phytopure DNA extraction kit (Amersham Pharmacia Biotech, Buckinghamshire, England), followed by a 1:1 (v/v) chloroform: phenol purification step.

DNA amplification

Genomic DNA was double-digested with a mixture of two restriction endonucleases, *Eco*RI and *Mse*I, for 2 h at 37 °C. Following heat inactivation of enzymes, *Eco*RI and *Mse*I adapters were ligated to digested DNA fragments to generate template DNA for amplification. These DNA fragments were then selectively amplified with an AFLP pre-amplification primer mix obtained from Life Technologies Co. (Carlsbad, Calif.). Amplified fragments were then diluted using 1 part of DNA solution to 9 parts of double-deionized water, and stored at –20 °C. These DNA fragments were then used as templates for further amplification with a total of 48 primer combinations.

The reaction mixture for the final amplification contained 25 ng/μl of template DNA, 0.168 ng/μl of ^{33}P- labeled *Eco*RI primer, 6.255 ng/μl of unlabeled *Mse*I primer with dNTPs, 1 μl of 10 × PCR buffer, 15 mM of $MgCl_2$, 1 unit/μl of *Taq* DNA polymerase and 3.5 μl of sterile double-deionized water to a final volume of 10 μl. The reactions were set up in 96-well plates, and overlaid with parafilm. Amplification was conducted in a PT-200 thermal cycler (MJ Research, Inc., Watertown, Mass.), programmed for 30 s at 94 °C, 30 s at 65 °C and 1 min at 72 °C. The annealing temperature was lowered 1 °C during each of the first 12 cycles. Then, 23 cycles were performed for 30 s at 94 °C, 30 s at 56 °C and 1 min at 72 °C. The PCR products were cooled down to 4 °C and stored at this temperature.

To prepare amplification products for electrophoresis, 10 μl of loading dye (98% formamide, 10 mM of EDTA, bromophenol blue and xylene cyanol) was added to each well. The DNA was then denatured at 90 °C for 3 min, and immediately placed on ice to prevent re-annealing. A 2.5-μl volume of DNA was then loaded into each well of a 0.4-mm thick Long Ranger (FMC Bioproducts, Rockland, Me.) gel. A total of 60 ml of Long Ranger solution was needed to prepare a single gel, and each gel was made-up of 7.2-ml of 50% Long Ranger gel solution, 26 g of Urea, 25 μl of TEMED and 12 ml 5 × TBE buffer. Double-deionized water was added to bring the volume to 60 ml. The gel-solution was then vacuum-filtered before it was stored for later use in gel preparation. When the gel was ready to be cast, 500 μl of 10% ammonium persulfate was added to the solution to facilitate gelling.

Electrophoresis was conducted at constant power (60 W) until the bromophenol blue (faster dye) reached the bottom of the gel.

Table 1 Available pedigree information for New Guineas in the 'Celebrette' and 'Celebration' Series as well as selected morphological information. * Cultivars with the same 4-digit source code are full-siblings; NA: not available

Series	Cultivar	Code #	Flower color	Leaf color	Varie-gation	Ball Flora Plant source code	Parents
Cellebrette	Apple Blossom	1	Pink	Green	None	6918-1	NA
Cellebrette	Cherry Rose	2	Red	Green	None	237-3	693 × 3177Br
Cellebrette	Cherry	3	Red	Green	None	7031-87	NA
Cellebrette	Frost	4	White	Green	None	7759	505 × 'Paradise Moorea'
Cellebrette	Grape Crush	5	Purple	Green	None	6926-1	NA
Cellebrette	Hot Pink	6	Pink	Green	None	994-1	916 × 'Paradise Grenada'
Cellebrette	Hot Rose	7	Red	Green	None	7108-1	NA
Cellebrette	Lavender	8	Lavender	Green	None	1161-5	'Moorea' × 7510D
Cellebrette	Light Pink	9	Pink	Green	None	6854-1	NA
Cellebrette	Orchid	10	Pink	Green	None	7637	NA
Cellebrette	Orange Crush	11	Orange	Green	None	6939-7	NA
Cellebrette	Peach	12	Peach	Dark bronze	None	6990-31	418 × 347
Cellebrette	Pink Jewel	13	Pink	Green	None	NA	NA
Cellebrette	Scarlet	14	Red	Green	None	7053-125	NA
Cellebrette	Wild Plum	15	Purple	Green	None	7771	'Paradise Prepona' × 523
Celebration	Apricot	16	Peach	Green	None	B271	NA
Celebration	Blush Pink Improved	17	Pink	Green	None	1748-3	'Moorea' × 50-4
Celebration	Bonfire	18	Red	Dark bronze	None	N4087-3	NA
Celebration	Bright Coral	19	Coral	Green	None	N4397-22	NA
Celebration	Candy Pink	20	Pink	Light bronze	None	BFP	NA
Celebration	Cherry Star	21	Red	Green	None	NA	NA
Celebration	Cherry Red	22	Red	Green	None	6827-1	NA
Celebration	Deep Coral	23	Coral	Dark bronze	Variegated	6477-1	NA
Celebration	Deep Pink	24	Pink	Light bronze	None	793-3	NA
Celebration	Deep Red	25	Red	Green	None	6827-8	NA
Celebration	Electric Pink	26	Pink	Light bronze	None	N4397-4	NA
Celebration	Electric Rose	27	Red	Dark bronze	None	BFP	NA
Celebration	Lavender Glow	28	Lavender	Green	None	7754	NA
Celebration	Light Lavender Improved	29	Lavender	Green	None	603-1	'Paradise Moorea' open pollinated
Celebration	Light Salmon Improved	30	Salmon	Green	Variegated	9993	NA
Celebration	Lavender III	31	Lavender	Green	None	NA	NA
Celebration	Light Salmon	32	Salmon	Green	None	NA	NA
Celebration	Orange	33	Orange	Green	None	6923-8	NA
Celebration	Purple	34	Purple	Green	None	7053-153	NA
Celebration	Purple Star	35	Purple	Dark bronze	None	6914-1	NA
Celebration	Rose	36	Red	Green	None	6489-4	NA
Celebration	Rose Star	37	Red	Dark bronze	None	1031-1	'Paradise Guadelupe' × 1005
Celebration	RS-Rose	38	Red	Green	None	NA	NA
Celebration	Salmon	39	Salmon	Green	Variegated	3207-1	2597 × 'Toga'
Celebration	Sangria	40	Pink	Green	None	7851	NA
Celebration	White	41	White	Green	None	7614	'Paradise Moorea' × 713

Gels were then dried using a SGD 4050 slab gel drier (Savant, Farmingdale, N.Y.), and exposed to an X-ray film. Films were developed using a Futura 2000 K automatic X-ray film processor (Fischer Industries, Geneva, Ill.). Reproducibility among gels was checked by repeating the entire AFLP process with several primer combinations, and amplification results were compared for accuracy.

Data analysis

X-ray films of different primer combinations were randomly selected for visual analysis, and 50 to 100 consecutive bands per primer combination were scored. Individual AFLP bands were scored as either present (1) or absent (0) on X-ray films. Very faint bands, along with bands present in all cultivars (i.e., monomorphic bands) and corresponding to highly repetitive sequences, were ignored. Bands corresponding to DNA fragments present in all individuals were ignored since they were uninformative. Since measures of genetic relatedness were relative, and not actual quantitative measures, it was unnecessary to include monomorphic bands, as they did not provide any information on the diversity of the population (Dudley, personal communication).

All data were analyzed using SAS (SAS Institute 2000), and following the procedures of Mumm et al. (1995). First, a dissimilarity matrix was constructed using Gower's Coefficient of Similarity (Gower 1971) whereby 0-0 matches were ignored when computing distances. A cluster analysis was then conducted to determine genetic relatedness among 41 cultivars. The Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) of cluster analysis was utilized. The cluster analysis was used to create a dendogram employing SAS.

Results

The AFLP analysis of all 41 cultivars generated between 73 and 130 scoreable polymorphic bands per primer combination (Table 2). Approximately 82% of scoreable bands were polymorphic between at least two of the cultivars analyzed (Fig. 1). This level of polymorphism was similar to that detected in other species, such as *Azadirachta* (Singh et al. 1999), daylily (Tomkins et al. 2001) and pecan (Vendrame et al. 1999), when the AFLP technique was used for measuring genetic variability. However, many of the non-polymorphic bands were very dark and wide, indicating that regions of the genome represented by these bands contained either high amounts of DNA or several DNA fragments of the same size. Since the amount of DNA contained in these regions of the genome could not be determined, the amount of genomic material present in these highly repetitive regions could not be estimated. Therefore, the level of polymorphism detected among New Guinea impatiens cultivars might be over-estimated. The high amount of DNA contained in these repetitive sequences would significantly lower the estimate of genetic diversity. Furthermore, since monomorphic bands were ignored in this analysis to facilitate computation, these estimates would most likely over-estimate the actual genetic diversity in this material (Barrett et al. 1998). However, this was not important, as measures of diversity were determined relative to other individuals in the population under consideration (Dudley, personal communication).

Table 2 Total number of scorable bands as well as percent polymorphic bands per primer combination

*Eco*RI Primer	*Mse*I Primer	Total number of bands	No. of polymorphic bands	% Polymorphic bands
1 (E-ACC)	1 (M-CAA)	93	76	81.7%
1 (E-ACC)	2 (M-CAC)	138	106	76.8%
2 (E-AAG)	1 (M-CAA)	137	119	86.9%
2 (E-AAG)	3 (M-CAG)	116	92	79.3%
3 (E-ACA)	6 (M-CTC)	157	130	82.8%
4 (E-ACT)	2 (M-CAC)	139	121	87.1%
4 (E-ACT)	3 (M-CAG)	100	85	85.0%
6 (E-ACG)	1 (M-CAA)	101	73	72.3%
6 (E-ACG)	3 (M-CAG)	129	113	87.6%
Total		1,110	915	
Average/primer combination		123.3	101.7	82.4%

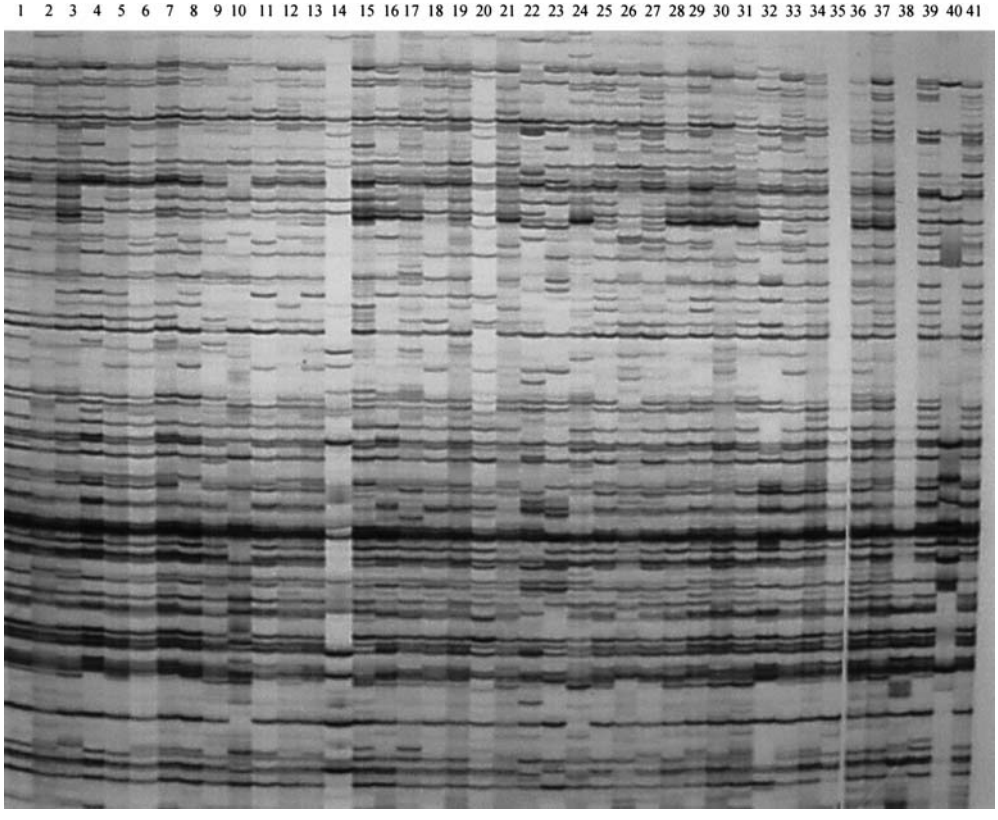

Fig. 1 AFLP profile of 41 cultivars of New Guinea impatiens created using *Eco*RI–3 (E-ACC) and *Mse*I–2 (M-CAC) primer combination. *Lanes 1 to 41* correspond to cultivars (code #1–41) listed in Table 1

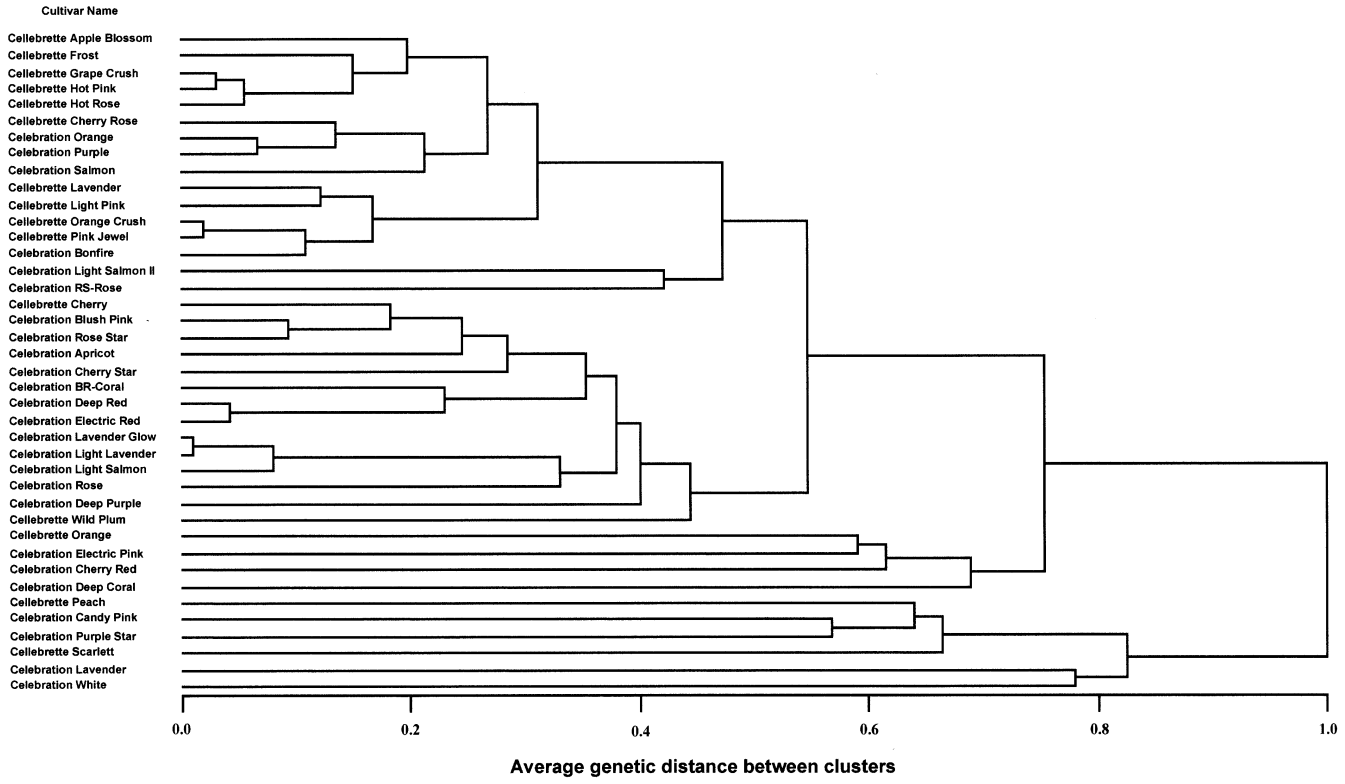

Fig. 2 Dendogram of New Guinea impatiens cultivars in the 'Cellebrette' and 'Celebration' series derived by the average linkage method (UPGMA) of cluster analysis

Gower's genetic dissimilarity estimates for the entire set of cultivars ranged from 0.9397 to 0.4875. The two most distantly related cultivars, based on the dissimilarity matrix, were 'Cellebrette Peach' and 'Celebration Lavender.' There were 12 other pairs of cultivars that were distantly related at an estimated distance greater than, or equal to, 0.8300. These pairs of cultivars were 'Cellebrette Scarlet' and 'Celebration Lavender'; 'Celebration Lavender' and 'Celebration Candy Pink'; 'Cellebrette Peach' and 'Celebration RS- Rose'; 'Celebration Orange' and 'Celebration White'; 'Cellebrette Hot Pink' and 'Celebration White'; 'Cellebrette Peach' and 'Celebration White'; 'Celebration Candy Pink' and 'Celebration White'; 'Celebration Cherry Rose' and 'Celebration White'; 'Celebration Deep Pink' and 'Celebration White'; 'Celebration Deep Red' and 'Celebration White'; 'Celebration Light Lavender' and 'Celebration White'; as well as 'Celebration Light Salmon' and 'Celebration White.'

'Celebration White' was most distantly related to most other cultivars. When compared to 33 of the other 40 cultivars, this cultivar had a Gower's dissimilarity estimate greater than 0.8000. Three of the most closely related cultivars to 'Celebration White' were 'Cellebrette Frost', 'Celebration Salmon' and 'Cellebrette Cherry Red.' Based on the pedigree information, 'Celebration White' and 'Celebrette Frost' shared a common ancestor, 'Paradise Moorea', and both had white flowers and similar foliage (Table 1).

Eight pairs of cultivars are related at a Euclidean distance of less than 0.5300. The two most closely-related cultivars, based on dissimilarity data, are 'Cellebrette Orange Crush' and 'Cellebrette Pink Jewel.' Pedigree information is unavailable for these cultivars, so no inferences can be drawn about this relationship. Other closely related cultivars include 'Cellebrette Frost' and 'Cellebrette Grape Crush'; 'Cellebrette Grape Crush' and 'Cellebrette Hot Pink'; 'Cellebrette Hot Pink' and 'Cellebrette Hot Rose'; 'Cellebrette Hot Rose' and 'Celebration Bonfire'; 'Celebration Bonfire' and 'Celebration Pink Jewel'; 'Celebration Deep Red' and 'Celebration Electric Rose'; and finally 'Celebration Orange' and 'Celebration Purple.'

Since the genetic relationships are expressed as Euclidean distances, it is possible for these relationships to be spatially represented (Mumm et al. 1994). The resulting dendogram established from the dissimilarity data has revealed several groupings of individuals within the population with similar genetic composition (Fig. 2). At a Euclidean distance of 0.5, there are four main groupings of cultivars. Interestingly, individuals within the 'Celebration' and 'Cellebrette' series grouped independently of each other, with some cultivars in one series being more closely-related to cultivars of the other series rather than with cultivars within the same series.

There were several groups of closely related cultivars at a Euclidean distance of less than 0.2. There were six

groups of plants observed, each group containing at least two cultivars (Fig. 2). In each of these groups, cultivars had similar morphological traits with a few minor exceptions (Table 1). Based on the dendogram, two of the groups contained five cultivars each. In one group, all of the cultivars, 'Cellebrette Apple Blossom', 'Cellebrette Frost', 'Cellebrette Grape Crush', 'Cellebrette Hot Pink' and 'Cellebrette Hot Rose' (Fig. 2), have the same green leaf color and lack of variegation (Table 1). In the other group, containing 'Cellebrette Lavender', 'Cellebrette Light Pink', 'Cellebrette Orange Crush', Cellebrette Pink Jewel' and 'Celebration Bonfire' (Fig. 2), only the last cultivar had a bronze leaf color, and unlike the remaining cultivars had green leaves (Table 1). However, similar to all other cultivars in this group, 'Celebration Bonfire' lacked variegated leaves (Table 1). Two of the groups contained three cultivars each (Fig. 2). One group, containing 'Cellebrette Cherry Rose', 'Celebration Orange' and 'Celebration Purple' (Fig. 2), exhibited green leaf color and lack of variegation in all cultivars (Table 1). In the second group, containing 'Cellebrette Cherry', 'Celebration Blush Pink' and 'Celebration Rose Star' (Fig. 2), all cultivars had green non-variegated leaves, except for 'Celebration Rose Star', which had dark bronze leaves (Table 1).

At a Euclidean distance of 0.2, there were two groups that contained only two cultivars (Fig. 2). Both 'Celebration Lavender Glow' and 'Celebration Light Lavender' (Fig. 2) had non-variegated green leaves (Table 1). The group of 'Celebration Deep Red' and 'Celebration Electric Red' (Fig. 2) also had non-variegated leaves, but 'Celebration Electric Red' had dark bronze leaves, while 'Celebration Deep Red' had green leaves (Table 1).

The most distantly related grouping of any two cultivars, based on the dendogram, included the pair of 'Celebration Lavender' and 'Celebration White.' These two cultivars were dissimilar to each other at a Euclidean distance greater than 0.75, and were dissimilar to all other cultivars at a distance greater than this estimate. The dendogram illustrated that these cultivars were more closely related to each other than any other cultivars, and they were both highly dissimilar to all other cultivars.

Discussion

The relatively high dissimilarity coefficients discovered in the New Guinea impatiens plant material under investigation indicate that a fairly high amount of diversity is present in the gene pool from which these cultivars are derived. The diversity of germplasm available for breeding efforts indicates that there is a good potential for genetic improvement of this species.

All primer combinations analyzed in this study showed high amounts of polymorphism in the New Guinea impatiens genome (Fig. 1). Overall, similar levels of polymorphisms were observed among all primer combinations tested (Fig. 1). Previous studies using AFLP markers in other plant species indicated that some primer combinations exhibited higher levels of polymorphism than others (Breyne et al. 1999; Yee et al. 1999; Garcia-Mas et al. 2000; Renganayaki et al. 2001). This finding indicated that the amount of genetic variability present in New Guinea impatiens was relatively high.

Currently, there is little knowledge about the genome of New Guinea impatiens, as it has not been adequately investigated. The AFLP technique is well suited for the analysis of unknown genomes (Tomkins et al. 2001) as it is more reproducible than other molecular marker systems, and AFLP profiles do not alter with minor variations in experimental conditions (Singh et al. 1999). In this study, the AFLP technique has proven to be useful in elucidating the genetic variation present in the New Guinea impatiens genome. The high level of observed polymorphisms with various primer combinations has indicated that a high amount of genetic diversity exists within this genome, even though the subset of cultivars included in this study are presumed to be derived from a narrow gene pool. Cytological studies have shown that different species of New Guinea impatiens have different basic chromosome numbers and ploidy levels (Arisumi 1974). This is a deterrent for genetic improvement of this species. Since the genetic background of the groups of New Guinea impatiens cultivars included in this study is unavailable, the results of the genetic analysis obtained in this study can be very useful. Determining the level of genetic variation present in New Guinea impatiens provides opportunities for genetic improvement of this crop. Similar findings of genetic diversity have been observed in other plant species using AFLP or PCR-based methods (Lu et al. 1996; Tomkins et al. 2001).

The pedigree information available for the New Guinea impatiens cultivars used in this study is limited, thus it is difficult to compare estimates of genetic diversity obtained through the AFLP technique to pedigree information. Studies comparing marker data to pedigree information have reported that marker data generally provide useful information for determining genetic relationships among species. Barrett et al. (1998) have compared AFLP-based genetic diversity estimates to pedigree information in wheat, and determined that AFLP-based estimates are reliable. However, in another study, Schut et al. (1997) have compared genetic relationships in barley based on AFLP markers, pedigree data and morphological traits, and have reported that the AFLP method is only moderately dependable for obtaining genetic diversity estimates. Degani et al. (2001) have in fact reported that RAPD-based estimates of genetic similarity are better correlated to pedigree-based estimates than AFLP-based estimates. However, the low reproducibility of RAPDs limits their effectiveness for molecular applications (Karp et al. 1997).

The relatively narrow genetic background of the cultivars used in this study has made it difficult to determine whether or not groupings obtained from the dendogram are in agreement with the limited pedigree information available. For instance, based on the limited pedigree information, cultivars 'Celebration Deep Red'

and 'Celebration Cherry Red' are full-siblings. However the dendogram does not reveal that these two cultivars are more closely-related than other cultivars. Likewise, 'Celebrette Scarlet', a full-sib of 'Celebration Purple', and 'Celebration Bright Coral', a full-sib of 'Celebration Electric Pink', are no more closely-related than all other cultivars, as demonstrated by the dendogram (Fig. 2). Lu et al. (1996) have reported that the AFLP technique could not distinguish between near-isogenic lines of pea when compared to both RAPD and SSR techniques. This may be due to the overall high level of polymorphism observed with the AFLP analysis. However, it has been suggested that AFLPs and other marker data could most likely provide better measurements of actual DNA differences within a group of genotypes than pedigree information, as both the selection pressure and the genetic drift are better accounted for (Barrett et al. 1998).

In this study, the only relationship that can be inferred from the available information is whether or not any two individuals are full-siblings. The identity of the parents of most cultivars used in this study is unknown, as breeding records have not been properly maintained (K. Strope, Ball FloraPlant, personal communication), except for 'Celebration Salmon' where genetic information has extended back for more than two generations. Based on the pedigree of this cultivar, it is evident that the cultivar 'Celebration Apricot' is in its lineage. However, this relationship is not revealed by the dendogram (Fig. 2).

Based on all six groupings made, the same variegation pattern (or lack thereof) is observed within each group that has been identified, but in three of the groups, a single cultivar having a different leaf color from the rest of the group has been noted. Overall, few inferences can be drawn about flower color. A gradient of flower colors is present in this material ranging from white to red, with minor differences in flower color observed among cultivars. Cultivars in smaller groupings, containing three or fewer cultivars, have exhibited similar flower color in three of the four small groupings identified. However, this may be a coincidence, as cultivars in larger groupings have a much wider range of flower colors (Table 1).

Although the background of the cultivars under investigation is unavailable, it is possible that closely related groups of cultivars have been derived from plant introductions that were obtained from close geographic regions. Cultivars derived from species that have evolved in close proximity to each other is a likely explanation for some of the groups that have been revealed by the dendogram in this study.

The information generated in this study, coupled with future planned studies for identifying AFLP markers linked to important phenotypic traits, will provide valuable information that can be used in breeding efforts for the improvement of New Guinea impatiens.

Acknowledgements The authors are grateful to Ms. Kerry Strope at Ball FloraPlant for plant material and other assistance. This project was supported by funds from the Office of Research of the University of Illinois Project no. 62–325.

References

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet 98:1320–1328

Arisumi TI (1973) Chromosome numbers and interspecific hybrids among New Guinea impatiens species. J Hered 64:77–79

Arisumi TI (1974) Chromosome numbers and breeding behavior of hybrids among Celebes, Java, and New Guinea species of *Impatiens* L. HortScience 9:478–479

Arisumi TI (1982) Endosperm balance numbers among New Guinea – Indonesian *Impatiens* species. J Hered 73:240–242

Ball V (1991) Ball redbook: greenhouse growing (15th edn) Geo J Ball Pub, West Chicago, Illinois

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci 38:1261–127

Barrett BA, Kidwell KK, Fox PN (1998) AFLP vs pedigree-based genetic diversity assessment methods. Crop Sci 38:1271–1278

Bretting PK, Widrelechner WP (1995) Genetic markers and plant genetic resource management. Plant Breed Rev 13:11–86

Breyne P, Boerjan W, Gerats T, Van Montagu M, Van Gysel A (1997) Applications of AFLP in plant breeding, molecular biology and genetics. Belgian J Bot 129:107–117

Breyne P, Rombaut D, Van Gysel A, Van Montagu M, Gerats T (1999) AFLP analysis of genetic diversity within and between *Arabidopsis thaliana* ecotypes. Mol Gen Genet 261:627–634

Degani C, Rowland LJ, Saunders JA, Hokanson SC, Ogden EL, Golan-Goldhirsh A, Galletta GJ (2001) A comparison of genetic relationship measures in strawberry (*Fragaria* × *ananassa* Duch.) based on AFLPs, RAPDs and pedigree data. Euphytica 117:1–12

Garcia-Mas J, Oliver M, Gomez-Paniagua H, de Vicente MC (2000) Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor Appl Genet 101:860–864

Gower JC (1971) A general coefficient of similarity and some of its properties. Biometrics 27:857–874

Karp A, Edwards K, Bruford M, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P, Arctander P, Tautz D, Hewitt G (1997) Newer molecular technologies for biodiversity evaluation: opportunities and challenges. Naturs Biotech 15:625–628

Lee M (1995) DNA markers and plant breeding programs. Adv Agron 55:265–344

Lin JJ, Kuo J, Ma J, Saunders JA, Beard HS, MacDonald MH, Kenworthy W, Ude GN, Matthews BF (1996) Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA techniques. Plant Mol Biol Rep 14:156–169

Lu J, Knox MR, Ambrose MJ, Brown JKM, Ellis THN (1996) Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. Theor Appl Genet 93:1103–1111

Martin T (1984) New Guinea impatiens. Horticulture 63:32–36

Mumm RH, Dudley JW (1994) A classification of 148 U.S. maize inbreds. I. cluster analysis based on RFLPs. Crop Sci 34:842–851

Mumm RH, Hubert LJ, Dudley JW (1994) A classification of 148 U.S. maize inbreds. II. Validation of cluster analysis based on RFLPs. Crop Sci 34:842–851

Mumm RH, Dudley JW (1995) A PC SAS computer program to generate a dissimilarity matrix for cluster analysis. Crop Sci 35:925–927

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Renganayaki K, Read JC, Fritz AK (2001) Genetic diversity among Texas bluegrass genotypes (*Poa arachnifera* Torr.) revealed by AFLP and RAPD markers. Theor Appl Genet 102:1037–1045

SAS Institute (2000) SAS online doc version 8

Schut W, Qi X, Stam P (1997) Association between relationship measures based on AFLP markers, pedigree data, and morphological traits in barley. Theor Appl Genet 95:1161–1168

Singh A, Negi MS, Rajagopal J, Bhatia S, Tomar UK, Srivastava PS, Lakshimikumaran M (1999) Assessment of genetic diversity in *Asadirachta indica* using AFLP markers. Theor Appl Genet 99:272–279

Tomkins JP, Wood TC, Barnes LS, Westman A, Wing RA (2001) Evaluation of genetic variation in daylily (*Hemerocallis* spp.) using AFLP markers. Theor Appl Genet 102:489–496

Vendrame WA, Kochert G, Wetzstein HY (1999) AFLP analysis of variation in pecan somatic embryos. Plant Cell Rep 28:853–857

Virk PS, Newbury HJ, Jackson MT, Ford-Lloyd BV (2000) Are mapped markers more useful for assessing genetic diversity? Theor Appl Genet 100:607–613

Yee E, Kidwell KK, Sills GR, Lumpkin TA (1999) Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of AFLP and RAPD markers. Crop Sci 39:268–275