

## Genetic Relationships of *Aglaonema* Species and Cultivars Inferred from AFLP Markers

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- **Background and Aims** *Aglaonema* is an important ornamental foliage plant genus, but genetic relationships among its species and cultivars have not been reported. This study analysed genetic relatedness of 54 cultivars derived from nine species using amplified fragment length polymorphism (AFLP) markers.
  - **Methods** Initially, 48 *EcoRI* + 2/*MseI* + 3 primer set combinations were screened, from which six primer sets that showed clear scoreable and highly polymorphic fragments were selected and used for AFLP reactions. AFLP fragments were scored and entered into a binary data matrix as discrete variables. Jaccard's coefficient of similarity was calculated for all pair-wise comparisons among the 54 cultivars, and a dendrogram was constructed by the unweighted pair-group method using the arithmetic average (UPGMA).
  - **Key Results** The number of AFLP fragments generated per primer set ranged from 59 to 112 with fragment sizes varying from 50 to 565 bp. A total of 449 AFLP fragments was detected, of which 314 were polymorphic (70 %). All cultivars were clearly differentiated by their AFLP fingerprints. The 54 cultivars were divided into seven clusters; cultivars within each cluster generally share similar morphological characteristics. Cluster I contains 35 cultivars, most of them are interspecific hybrids developed mainly from *A. commutatum*, *A. crispum* or *A. nitidum*. However, Jaccard's similarity coefficients among these hybrids are 0.84 or higher, suggesting that these popular hybrid cultivars are genetically much closer than previously thought. This genetic similarity may imply that *A. nitidum* and *A. crispum* are likely progenitors of *A. commutatum*.
  - **Conclusions** Results of this study demonstrate the efficiency and ease of using AFLP markers for investigating genetic relationships of ornamental foliage plants, a group usually propagated vegetatively. The AFLP markers developed will help future *Aglaonema* cultivar identification, germplasm conservation and new cultivar development.
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**Key words:** AFLP markers, Araceae, *Aglaonema*, Chinese evergreens, genetic variation, ornamental tropical foliage plants.

### INTRODUCTION

The genus *Aglaonema* Schott belongs to the family Araceae Juss. and comprises 21 species (Nicolson, 1969). All species are herbaceous evergreens native to south-east Asia, north-eastern India, across southern China, and into Indonesia and New Guinea where they inhabit humid and heavily shaded tropical forests (Nicolson, 1969; Mayo *et al.*, 1997). Flowers are unisexual, dichogamous in nature and, thus, most species are open-pollinated. The chromosome number varies from  $2n = 42$  to 60 or 120 (Nicolson, 1969) depending on species. Due to their attractive foliar variegation and tolerance to low light, *Aglaonema* species have been cultivated in China and other Asian countries for centuries as indoor ornamental foliage plants or houseplants. As a result, *Aglaonema* are commonly referred to as Chinese evergreens. Chinese evergreens were introduced into the Royal Botanic Gardens, Kew in 1885 (Brown, 1885).

*Aglaonema* cultivation in the USA started in the 1930s (Smith and Scarborough, 1981) and soon became a great

success in Florida, where most foliage plants are now produced. The success can be partly attributed to an increasing release of attractive interspecific hybrids (Chen *et al.*, 2002) as the number of commercial cultivars grown in Florida increased from ten in 1975 to more than 50 in 2002. Many of the cultivars have gained wide recognition and contribute significantly to the ornamental foliage plant industry. For example, 'Silver Queen', a hybrid selected from a cross between *A. commutatum* Schott 'Treibii' and *A. nitidum* (Jack) Kunth 'Curtisii', brings about \$4 million to the Florida state economy annually (Elliott *et al.*, 1998). With the discovery of new *Aglaonema* species in the Philippines and Thailand in the 1970s (Brown, 2001), wild collections as well as new hybrids have been introduced to Florida. A total of 29 *Aglaonema* hybrids received USA patents during the 1990s (Henny and Chen, 2003). Genetic information on some of these hybrids, however, is unclear.

Although *Aglaonema* has become an important ornamental foliage genus, neither the US National Plant Germplasm System (NPGS) nor the International Board for Plant Genetic Resources (IBPGR) had been actively involved in conservation of *Aglaonema* or other foliage plant germ-

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plasm until recently. The Ornamental Plant Germplasm Center (OPGC) established in 1999 at Ohio State University is considering conservation of five genera from the family Araceae including *Aglaonema*. Currently, most *Aglaonema* resources have been collected and maintained by private plant collectors or public institutions such as botanical gardens or conservatories (Henny and Chen, 2003). Because of the expense of maintaining tender tropical plants in glasshouses, it is conceivable that changes in funding or in individual research interests could result in a loss of plant diversity in these invaluable collections.

Scientific conservation and utilization of genetic resources require accurate information on genetic relatedness among species and cultivars. However, attempts to classify *Aglaonema* species based on morphology have been difficult and controversial. In his monograph *A Revision of the Genus Aglaonema (Araceae)*, Nicolson (1969) identified 21 species. The monumental *Hortus III* (Bailey and Bailey, 1976) described ten species, Jervis (1980) identified 17 species, and the *New Royal Horticultural Society Dictionary of Gardening* documented 11 species (Huxley, 1994). Among the species, the origin of *A. commutatum* has been at the centre of controversy due to its morphological variability. Nicolson (1969) hypothesized that *A. commutatum* might be of hybrid origin since it shares some morphological characters with *A. simplex* Blume, *A. nitidum* and *A. marantifolium* Blume. Later, Jervis (1980) proposed that *A. commutatum* might originate from introgressive hybridization of *A. crispum* (Pitcher & Manda) Nicols., *A. simplex* and *A. philippense* Engl. However, neither of these two hypotheses has been tested.

Amplified fragment length polymorphism (AFLP) is a novel PCR-based assay for plant DNA fingerprinting that reveals significant levels of DNA polymorphism (Vos *et al.*, 1995). Molecular marker profiles based on AFLPs can be used to detect variation in the DNA level and have proved to be extremely effective in distinguishing closely related genotypes. AFLP has been used in the assessment of genetic relationships of a wide range of species, including some ornamentals such as *Caladium* Venten. spp. (Loh *et al.*, 1999, 2000), *Hemerocallis* L. spp. (Tomkins *et al.*, 2001), *Musa acuminata* Colla (Wong *et al.*, 2001), *Impatiens hawkeri* W. Bull. (Carr *et al.*, 2003), *Orchis simia* Vill. (Qamaruz-Zaman *et al.*, 1998) and *Pelargonium peltatum* (L.) L'Her. ex Ait. (Barcaccia *et al.*, 1999). However, genetic relationships of *Aglaonema* species and cultivars at DNA levels have not been determined thus far. The objectives of this study were to assess the usefulness of AFLP for cultivar identification and to determine genetic relationships of common *Aglaonema* species and cultivars.

## MATERIALS AND METHODS

### Plant material

Fifty-four *Aglaonema* cultivars, along with one cultivar each of five other ornamental aroid genera (*Alocasia* (Schott) G. Don., *Anthurium* Schott, *Dieffenbachia* Schott, *Philodendron* Schott and *Spathiphyllum* Schott), were collected from either the research glasshouses at the Mid-

Florida Research and Education Center (MREC), University of Florida or ornamental foliage plant nurseries in central Florida (Table 1).

### Fluorescent-AFLP analysis

Total DNA was extracted from young leaves of 60 samples (54 *Aglaonema* cultivars, one duplicated *A. commutatum* 'Treubii', and five other aroid genera) using the DNeasy system (Qiagen, Valencia, CA, USA). After DNA quantification using a Hoefer DyNA Quant 200 (Pharmacia Biotech, Piscataway, NJ, USA), AFLP analysis was conducted using the GIBCO BRL AFLP System II (Life Technologies, Grand Island, NY, USA) and visualized with the LI-COR IR automated sequencer 4000-L (Li-COR Inc., Lincoln, NE, USA). Total DNA (125 ng) from all 60 samples was digested with 1  $\mu$ l of a mixture of *EcoRI/MseI* (1.25 units  $\mu$ l<sup>-1</sup>) at 37 °C overnight and ligated to *EcoRI/MseI* adapters with 1.5  $\mu$ l (1 unit  $\mu$ l<sup>-1</sup>) of T4 DNA ligase at 25 °C for at least 6 h. The adaptor-ligated DNA was amplified using a mixture of 2.5  $\mu$ l of DNA from the ligation reaction, 20  $\mu$ l of pre-amplification mix II, 2.5  $\mu$ l of 10  $\times$  PCR buffer, and 0.2  $\mu$ l of *Taq* DNA polymerase (5 units  $\mu$ l<sup>-1</sup>). The pre-amplification reactions were performed on a MJR Cycle LR™ (MJ Research, Inc., Watertown, MA, USA) using the following cycling parameters: 30 cycles at 94 °C for 15 s, 56 °C for 30 s plus 1 extra s per cycle, and 72 °C for 1 min plus 1 extra s per cycle, then 1 cycle at 72 °C for 3 min. The pre-amplified PCR product was quantified in the fluorometer, and the amount of template for the subsequent PCR was diluted to 125  $\mu$ g  $\mu$ l<sup>-1</sup>. Selective amplification was performed using a reaction mix composed of 2  $\mu$ l of DNA from pre-amplification, 2  $\mu$ l of *MseI* primer (8.3  $\mu$ M), 0.5  $\mu$ l of IRD700-labeled *EcoRI* primer (1  $\mu$ M), 0.5  $\mu$ l of IRD800-labeled *EcoRI* primer (1  $\mu$ M), 1  $\mu$ l of 10  $\times$  PCR buffer, 4  $\mu$ l of H<sub>2</sub>O, and 0.16  $\mu$ l of *Taq* DNA polymerase (5 units  $\mu$ l<sup>-1</sup>).

The selective amplification PCRs were performed as follows: 13 cycles at 94 °C for 15 s, 65 °C for 30 s minus 0.7 s per cycle, and 72 °C for 1 min, then 30 cycles at 94 °C for 15 s, 56 °C for 30 s plus 1 extra s per cycle, 72 °C for 1 min plus 1 extra s per cycle, then 72 °C for 3 min. Both pre- and selective-amplification conditions were modified according to Myburg *et al.* (2000). The products from the selective amplification were electrophoresed on 25 cm  $\times$  0.25 mm 8 % denaturing polyacrylamide Long Ranger® Gel Solution (BMA, Rockland, ME, USA) in 0.8  $\times$  TBE buffer using a LI-COR automated sequencer 4000-L. The gel was pre-run for 10–20 min at 1500 V, 40 mA, and 40 W until the gel temperature reached 50 °C. The samples were denatured at 95 °C for 3 min and immediately placed on ice. Electrophoresis was performed at 1500 V, 50 °C for 3.5 h after 1–15  $\mu$ l samples and 1  $\mu$ l of a mixture of IRD700 and IRD800 size markers were loaded (Li-COR Inc., Lincoln, NE, USA).

A total of 48 AFLP primers were initially screened, from which six primer sets (IRD700 E + TC/M + CAA; IRD800 E + AA/M + CAA; IRD700 E + TC/M + CAT; IRD800 E + AA/M + CAT; IRD700 E + GG/M + CAA; and IRD800 E + CC/M + CAA) that showed clear scoreable and also highly

TABLE 1. *List of 54 Aglaonema cultivars and five other aroid genera used in this study*

Cultivars	Background	Sources
Treubii	<i>A. commutatum</i>	UF/MREC*
Curtissi	<i>A. nitidum</i>	UF/MREC
Ernesto's Favorite	<i>A. nitidum</i>	UF/MREC
Silver Queen	Interspecific hybrid	UF/MREC
277028	Interspecific hybrid	UF/MREC
Emerald Bay	Somaclonal variant	UF/MREC
Golden Bay	Interspecific hybrid	UF/MREC
Moonlight Bay	Somaclonal variant	UF/MREC
Diamond Bay	Somaclonal variant	UF/MREC
257-12	Interspecific hybrid	UF/MREC
Echo	<i>A. commutatum</i>	UF/MREC
Cory	Interspecific hybrid	UF/MREC
Costatum Hybrid	Unknown	Twyford International, Inc.†
Queen of Siam	Interspecific hybrid	Stewart's Greenhouse, Inc.‡
White Rain	Hybrid	Stewart's Greenhouse, Inc.
Stripes	Hybrid	UF/MREC
White Lance	Hybrid	Stewart's Greenhouse, Inc.
Green Lady	Interspecific hybrid	Stewart's Greenhouse, Inc.
Jubilee	Hybrid	Green Star Foliage, Inc.§
Jubilee Petite	Hybrid	Stewart's Greenhouse
Maria Christina	Unknown	Stewart's Greenhouse
Painted Princess	Hybrid	Stewart's Greenhouse
J.G.	Hybrid	UF/MREC
1502	Hybrid	UF/MREC
Amelia	Hybrid	Stewart's Greenhouse
B.J. Freeman	<i>A. crispum</i>	Stewart's Greenhouse
Camouflage	Hybrid	Stewart's Greenhouse
Jewel of India	Interspecific hybrid	Green Star Foliage
Maria	Unknown	Green Star Foliage
Mary Ann	Hybrid	Stewart's Greenhouse
Patricia	Hybrid	Stewart's Greenhouse
Royal Ripple	Hybrid	Stewart's Greenhouse
Silver Ribbon	Hybrid	Stewart's Greenhouse
Silver Bay	Interspecific hybrid	UF/MREC
Commatum	Unknown	UF/MREC
Deborah	Sport	Green Star Foliage
Emerald Star	Interspecific hybrid	Green Star Foliage
Stars	Interspecific hybrid	Stewart's Greenhouse
Donna Carmen Mutant	Sport	Twyford International, Inc.
Peacock	Interspecific hybrid	Stewart's Greenhouse
III	Unknown	Twyford International, Inc.
JT2000	Unknown	Stewart's Greenhouse
Pride of Sumatra	Interspecific hybrid	Twyford Inc.
Red Gold	Interspecific hybrid	Twyford international, Inc.
Donna Carmen	Interspecific hybrid	Stewart's Greenhouse
Modestum	<i>A. modestum</i>	UF/MREC
714-12	Unknown	UF/MREC
Mod × Rot Clone 1	Interspecific hybrid	Twyford Inc.
Mod × Rot Clone 2	Interspecific hybrid	Twyford Inc.
Mod × Rot Clone 3	Interspecific hybrid	Twyford Inc.
Mod × Rot Clone 4	Interspecific hybrid	Twyford Inc.
Pictum Tricolor	<i>A. pictum</i>	UF/MREC
XII	Unknown	Twyford Inc.
XIV	Unknown	Twyford Inc.
Red Hot	<i>Anthurium</i> ×	UF/MREC
64706	<i>Spathiphyllum</i> ×	UF/MREC
Moonlight	<i>Philodendron</i> ×	Agri-Starts, Inc.¶
Compacta	<i>Dieffenbachia maculata</i> 'Compacta'	UF/MREC
Polly	<i>Alocasia</i> ×	Agri-Starts, Inc.

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† Twyford International, Inc. Apopka, FL, USA.

‡ Stewart's Greenhouse, Inc. Mt Dora, FL, USA.

§ Green Star Foliage, Inc. Apopka, FL, USA.

¶ Agri-Starts, Inc. Apopka, FL, USA

polymorphic fragments were selected for fluorescent-AFLP reactions with the samples of the *Aglaonema* cultivars and five other aroid genera.

#### Data analysis

For the genetic similarity analysis, AFLP fragments were visually scored as present (1) or absent (0) to create a binary data set. The data was entered into a binary data matrix as discrete variables. Jaccard's coefficient of similarity (Sneath and Sokal, 1973) was calculated for all pair-wise comparisons among the *Aglaonema* cultivars and five other aroid genera as follows:  $Jaccard = N_{AB} / (N_{AB} + N_A + N_B)$ , where  $N_{AB}$  is the number of fragments shared by two cultivars (A and B),  $N_A$  represents amplified fragments in cultivar A and  $N_B$  represents fragments in cultivar B. A dendrogram was constructed using NTSYS version 2.1 (Exeter Software, Setauket, NY, USA) (Rohlf, 2000) based on the unweighted pair group method of the arithmetic average (UPGMA).

## RESULTS AND DISCUSSION

#### AFLP profiles and analysis

The six primer sets generated clear-cut AFLP profiles for the 54 *Aglaonema* cultivars and five other aroid genera. The duplicate cultivar 'Trebii' showed identical AFLP patterns with each primer set. An example of fluorescent-AFLP profiles of 30 *Aglaonema* cultivars with five other aroid genera using primer IRD700 E + GG/M + CAA is shown in Fig. 1. In a theoretical consideration of the number of primers needed to produce accurate estimates of genetic relatedness, Ellis *et al.* (1997) believed that by choosing the six best combinations of primers, it is possible to explain more than 80 % of the expected relatedness. Using six primer combinations selected from our initial screening of 48, it was found that the AFLP fragments ranged from 50 to 565 bp, with a majority of the polymorphism distributed between 150 and 350 bp. A total of 449 fragments were scored, of which 314 (70 %) were polymorphic (Table 2), suggesting that each primer generated an average of 74.8 fragments and 52.3 of them were polymorphic. The level of polymorphism was similar to that detected in other plants when the AFLP technique was used for measuring genetic variability. Aggarwal *et al.* (2002) identified 501 AFLP markers from Basmati rice (*Oryza sativa* L.) with 65 % of them being polymorphic. Singh *et al.* (1999) reported a total of 422 fragments were obtained from *Azadiracht indica* A. Juss, of which 297 (70 %) were polymorphic. Tomkins *et al.* (2001) determined that the average percentage of poly-

morphism was 79 % per primer combination in daylily (*Heimerocallis* spp).

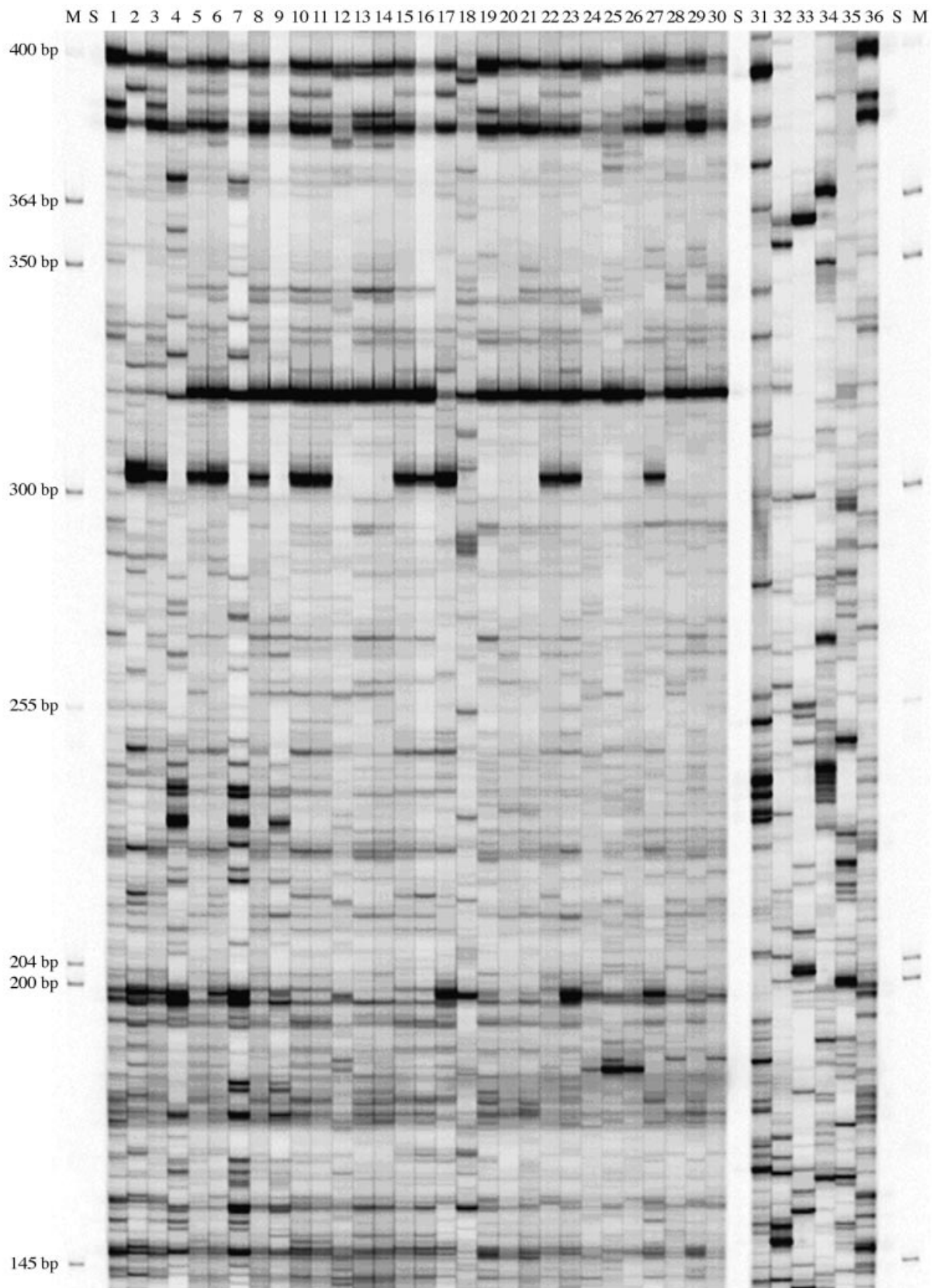
#### Genetic relationships among cultivars

Based on the UPGMA analysis, a dendrogram for the *Aglaonema* cultivars and also other five aroid genera was constructed (Fig. 2). Fifty-four *Aglaonema* cultivars were grouped into seven clusters. Cluster I is the largest, comprising 35 cultivars which can be divided into four subclusters, IA, IB, IC and ID. Subcluster IA includes *A. commutatum* 'Trebii' only, and IB includes *A. nitidum* 'Curtisii' and *A. nitidum* 'Ernesto's Favorite'. *Aglaonema commutatum* generally displays foliar variegation confluent in bars along the primary lateral veins of the leaf, rarely in irregularly scattered spots (Nicolson, 1969). Foliar variegations of *A. nitidum* include narrow, silvery stripes along lateral veins or silvery blotches around the midrib.

Subcluster IC contains 19 cultivars, which can be further broken into four groups. The cultivar in the first group is 'Silver Queen', an interspecific hybrid selected from a cross of *A. commutatum* 'Trebii' and *A. nitidum* 'Curtisii'. The second group includes an interspecific hybrid '277028', developed from a cross between *A. commutatum* 'Trebii' and *A. nitidum* 'Ernesto's Favorite'. 'Emerald Bay' is a somaclonal variant selected from 'Golden Bay' (Henny *et al.*, 2003). 'Golden Bay' was derived from a cross of '277028' and 'MREC 1501' (Henny and Chen, 2001). The 'MREC 1501' was a hybrid selected from a cross of *A. commutatum* 'Tricolor' and *A. × Manila*. Whereas *Aglaonema × Manila* was collected from the Philippines in 1962 and believed to be a hybrid resulting from a cross of *A. commutatum* 'Tricolor' with an unknown species (Brown, 2001). 'MREC 1501' and *A. × Manila* were not included in this study as they were not available. Both 'Moonlight Bay' and 'Diamond Bay' are somaclonal variants selected from 'Silver Bay', which was a hybrid of *A. × Manila Whirl* and *A. nitidum* 'Ernesto's Favorite' (Henny *et al.*, 1992). Cultivar '257-12' was developed from a cross of '1502' and *A. nitidum* 'Curtisii'. Another cultivar, 'Echo', also belongs to the second group. 'Echo' was found in Manila, the Philippines (Jervis, 1980) and is a cultivar of *A. commutatum*.

There are six cultivars in the third group of IC. 'Cory' is a hybrid selected from a cross of *A. nitidum* 'Curtisii' with an unknown plant. 'Costatum hybrid', which has a Jaccard's coefficient of 0.96 with 'Cory', is most likely not a cultivar from *A. costatum* Schott since cultivars related to *A. costatum* are positioned in cluster II. 'Queen of Siam' is an interspecific hybrid selected from *A. commutatum*

FIG. 1. Fluorescent-AFLP profiles of 30 *Aglaonema* cultivars, five cultivars from other aroid genera, and a duplicate *A. commutatum* 'Trebii' using primer IRD700 E + GG/M + CAA. The samples are arranged from left to the right in the order of (M) size markers, (S) space, (1) 'Trebii', (2) 'Curtisii', (3) 'Silver Queen', (4) 'Modestum', (5) '277028', (6) '257-12', (7) '714-12', (8) 'Echo', (9) 'Peacock', (10) 'Emerald Bay', (11) 'Golden Bay', (12) 'Donna Carmen', (13) 'J.G.', (14) '1502', (15) 'Moonlight Bay', (16) 'Diamond Bay', (17) 'Ernesto's Favorite', (18) 'Pictum Tricolor', (19) 'Amelia', (20) 'B.J. Freeman', (21) 'Camouflage', (22) 'Cory', (23) 'Costatum Hybrid', (24) 'Deborah', (25) 'Donna Carmen Mutant', (26) 'Emerald Star', (27) 'Green Lady', (28) 'III', (29) 'Jewel of India', (30) 'JT2000', (S) space, (31) *Anthurium* 'Red Hot', (32) *Spathiphyllum* '64760', (33) *Philodendron* 'Moonlight', (34) *Dieffenbachia* 'Compacta', (35) *Alocasia* 'Polly', (36) *Aglaonema* 'Trebii', (S) space, and (M) size markers.



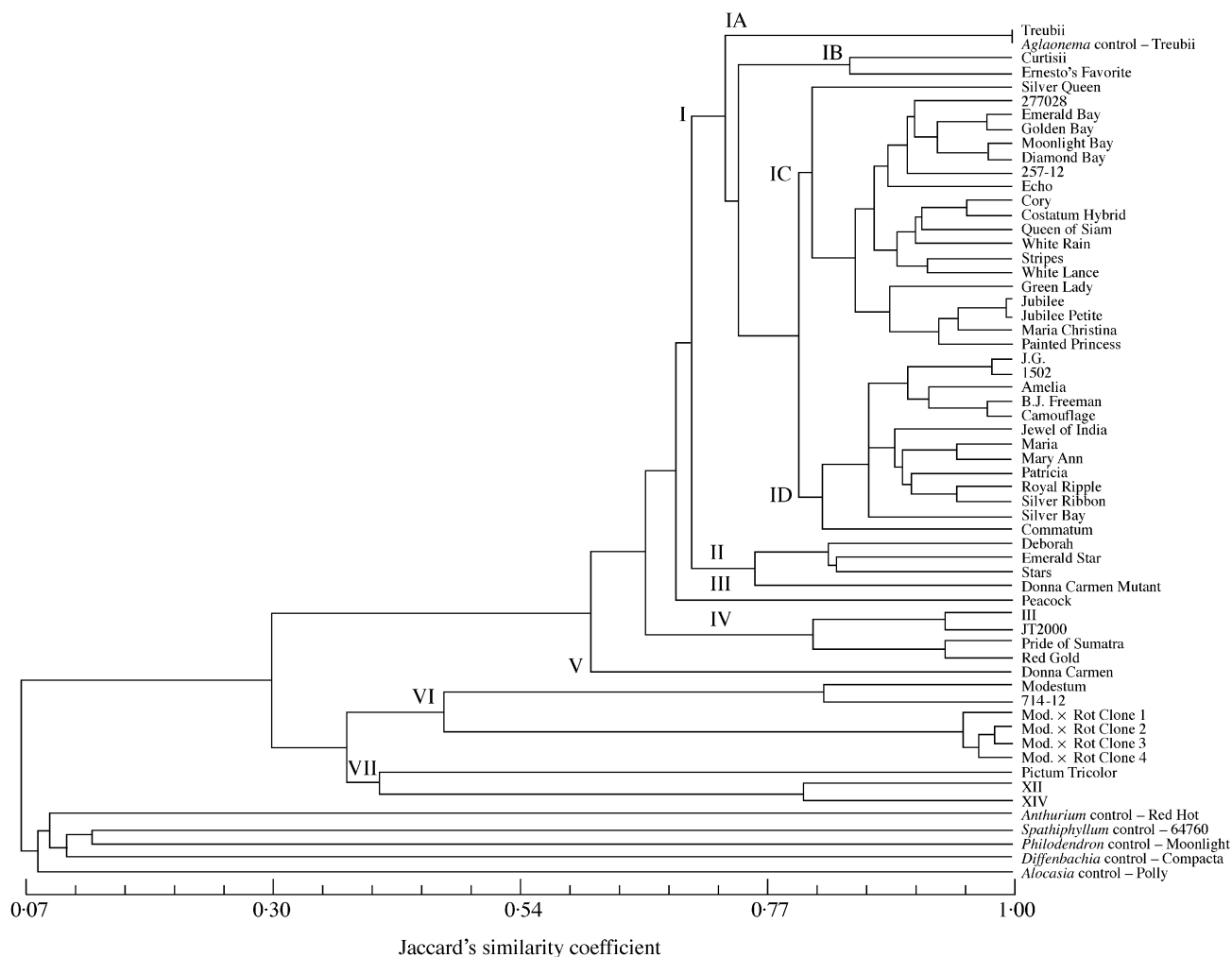


FIG. 2. Dendrogram of 54 *Aglaonema* cultivars, a duplicate *A. commutatum* 'Treubii', and five cultivars from other aroid genera resulting from a UPGMA cluster analysis based on Jaccard estimates of similarity obtained from 449 polymorphic AFLP bands.

TABLE 2. AFLP primer combinations, primer sequences, total number of bands generated by each primer set, number of polymorphic bands detected, and percentages of polymorphic bands used in the study of *Aglaonema* cultivars

Primer	Total no. of bands	No. of polymorphic bands	% of polymorphic bands
IRD700 E + TC/M + CAA	69	43	62
IRD800 E + AA/M + CAA	66	45	68
IRD700 E + TC/M + CAT	74	54	73
IRD800 E + AA/M + CAT	69	46	67
IRD700 E + GG/M + CAA	112	86	77
IRD800 E + CC/M + CAA	59	40	68
Total	449	314	70
Average	74.8	52.3	70

'Tricolor' and *A. nitidum* 'Ernesto's Favorite'. 'White Rain' is a hybrid of *A. nitidum* 'Ernesto's Favorite' crossed with *A. cv.* 'Panayensis'. There is no species information available about *A. cv.* 'Panayensis', and this cultivar was not used in this study. 'Stripes' is a hybrid of *A. 'Manila'* and *A. nitidum* 'Curtisii'. 'White Lance' is a hybrid of *A. cv.* 'Bayonet' and

*A. nitidum* 'Ernesto's Favorite'. Again, *A. cv.* 'Bayonet' has an unknown origin and was not included in this study. There are five cultivars in the fourth group of IC. 'Green Lady', according to the USA plant patent no. 10271 (or PP10271), was selected from a cross between *A. commutatum* and *A. pictum* (Roxb.) Kunth. This claim is probably untrue

because *A. pictum* has ten unique fragments, of which none are present in 'Green Lady'. 'Jubilee' is a hybrid of *A. nitidum* 'Curtisii' and *A. × 'Manila'*, 'Jubilee Petite' a hybrid of *A. nitidum* 'Curtisii' and *A. commutatum* 'Tricolor'. The origin of 'Maria Christina' is not completely clear, but it could be a hybrid of *A. nitidum* and *A. commutatum* as it morphologically resembles 'Silver Queen'. 'Painted Princess' (USA PP9081) was claimed as a hybrid from unidentified parents. However, its parents could be related to *A. nitidum* and *A. commutatum* as well since it is genetically similar to the cultivars in the fourth group of IC (Jaccard's similarity coefficient = 0.90).

Cluster ID includes 13 cultivars, which fall into two groups. There are three subgroups in the first group. Both 'J.G.' and '1502' are hybrids selected from the same cross between *A. commutatum* 'Tricolor' and *A. × 'Manila'*. 'Amelia' (USA PP8977) is a hybrid without identified parents. 'B.J. Freeman' is a cultivar of *A. crispum*. 'Camouflage' is a hybrid developed from a cross of *A. × 'Manila'* and 'FB-23'. The origin of 'FB-23' is unknown; but it may be related to *A. crispum*. Due to its unavailability, 'FB-23' was not used in this study. A distinct feature of *A. crispum* is its 'ashy' variegation that completely covers the leaves except for the midvein and margin. The second subgroup includes 'Jewel of India', a hybrid of *A. commutatum* 'Malay Lady' and *A. crispum* 'Dow Hybrid'. 'Maria' has been a common cultivar in the foliage plant industry for decades, and it may have a relationship with *A. commutatum*. 'Mary Ann' (USA PP8976), 'Patricia' (USA PP10269) and 'Royal Ripple' (USA PP9076) all have no identified parents even though the USA patents were issued. Due to its genetic (Jaccard's similarity coefficient = 0.95) and morphological (bi-coloured leaves marked with silver-green on dark green background) similarity to 'Maria', 'Mary Ann' may be related to *A. commutatum*. The solid-silver colour between the midrib and leaf margin led us to believe that 'Patricia' may have genetic makeup similar to *A. crispum*. 'Silver Ripple' is both genetically and morphologically close to 'Silver Ribbon'. 'Silver Ribbon' was developed in India as a hybrid of *A. commutatum* var. 'Picturatum' and *A. commutatum* var. 'Silver III'. 'Silver Bay', a hybrid of *A. × 'Manila Whirl'* and *A. nitidum* 'Ernesto's Favorite' (Henny *et al.*, 1992) as mentioned previously, is the only cultivar in the third subgroup of ID subcluster. There is only one cultivar, 'Commatum', in the second group of ID, which may be a selection within *A. commutatum*.

Cluster II includes four cultivars. 'Deborah' is assumed to be a sport of 'Queen of Siam'. 'Emerald Star' is another hybrid introduced from India, it was selected from of a cross of *A. commutatum* 'Elegans' and *A. costatum*. Leaf characteristics of *A. costatum* include scattered white or yellow spots on both sides of leaves and a white midrib. 'Stars' is a hybrid of a cross of *A. commutatum* and *A. brevispathum* (Engl.) Engl. Foliar variegation of *A. brevispathum* is also characterized by small white and yellow spots. Since *A. brevispathum* and *A. costatum* are closely related (Nicolson, 1969), 'Emerald Star' and 'Stars' are genetically close and morphologically similar. 'Donna Carmen Mutant' is presumed to be a sport of 'Donna Carmen'. However, its

similarity to cultivars from either *A. brevispathum* or *A. costatum* raises a doubt about this presumption.

There is only one cultivar in cluster III – 'Peacock', which is a hybrid selected from a cross of *A. commutatum* 'Echo' and *A. marantifolium* Blume 'Tricolor'. *Aglaonema marantifolium* 'Tricolor' has narrow areas of mottled yellow and green extended along the midrib and lateral veins (Jervis, 1980). 'Peacock' leaves have a mottled green colour with random dark green spots and yellow green midribs.

Cluster IV contains four cultivars. There is no information available as to the origin and pedigree of 'III' and 'JT2000'. 'Pride of Sumatra' was from Indonesia and most likely relates to *A. rotundum* and *A. marantifolium* since its morphology resembles 'Red Gold'. 'Red Gold' is a hybrid developed by Sithiporn Donavanik of Thailand who crossed *A. rotundum* with *A. marantifolium* 'Tricolor'. *Aglaonema rotundum* has pink to red midribs and veins on a dark, glossy green upper surface. 'Red Gold' has red veins strongly resembling a *Codiaeum variegatum* and was later named *A. sithiporn*. Twyford Plant Laboratories, Inc., Sebring, Florida renamed it as 'Red Gold' (Brown, 2001).

Only one cultivar, 'Donna Carmen' is positioned in cluster V. This cultivar was developed by botanist Gregory Hambali of Bogar, Indonesia. It also has red leaves and resulted from crosses of *A. rotundum* with another, unknown, variety.

Cluster VI has *A. modestum* and hybrids of *A. modestum* crossed by other species. Based on the AFLP polymorphism, *A. modestum* is clearly separated from other *Aglaonema* species. This species has solid green leaves and has been cultivated in the East for centuries as a symbol of good fortune. The origin of '714-12' is unclear. The 'Mod. × Rot. Clone' series were believed to be crosses between *A. modestum* and *A. rotundum*.

Three cultivars are situated in cluster VII; subcluster VIIA has *A. pictum* 'Tricolor', and subcluster VIIB has cultivars 'XII' and 'XIV'. The Jaccard's similarity coefficient of *Aglaonema pictum* 'Tricolor' is the lowest (0.31) with all other *Aglaonema* species based on the AFLP polymorphism. Cultivars 'XII' and 'XIV' are of unknown origins. Their AFLP polymorphisms are different from other *Aglaonema* cultivars tested and their origins remain unclear.

Cultivars clustered based on the similarity of AFLP polymorphisms are also morphologically related. Variegation patterns of cultivars in cluster I include silvery stripes along the principle lateral veins, ash blotches on the centre of the leaf or between the midrib and margin, or irregular grey blotches adjacent to primary lateral veins. These variegation patterns are characteristics of *A. commutatum*, *A. crispum*, *A. nitidum* or hybrids derived from two of the three species (Henny, 1983, 1986). Foliar variegation patterns of cluster II cultivars most resemble *A. costatum* or *A. brevispathum* with scattered white or yellowish spots on both sides of the leaves. 'Peacock' in cluster III has a unique pattern of leaf variegation, characterized by a mottled green colour with random dark green spots. This pattern is probably a combination of *A. commutatum* 'Echo' and *A. marantifolium* 'Tricolor'. Cultivars in cluster IV and V are distinguished by red-veined leaves, which is the character-

TABLE 3. Jaccard's similarity coefficients among seven cultivars of *A. commutatum*, *A. crispum*, *A. modestum*, *A. nitidum* or *A. pictum* using six primer sets

	<i>A. commutatum</i> 'Echo'	<i>A. commutatum</i> 'Trebuii'	<i>A. crispum</i> 'B.J. Freeman'	<i>A. modestum</i>	<i>A. nitidum</i> 'Curtisii'	<i>A. nitidum</i> 'Ernesto's Favorite'	<i>A. pictum</i> 'Tricolor'
<i>A. commutatum</i> 'Echo'	–	0.69	0.79	0.40	0.72	0.75	0.32
<i>A. commutatum</i> 'Trebuii'	–	–	0.74	0.38	0.67	0.63	0.33
<i>A. crispum</i> 'B.J. Freeman'	–	–	–	0.42	0.66	0.70	0.33
<i>A. modestum</i>	–	–	–	–	0.37	0.38	0.31
<i>A. nitidum</i> 'Curtisii'	–	–	–	–	–	0.85	0.31
<i>A. nitidum</i> 'Ernesto's Favorite'	–	–	–	–	–	–	0.32
<i>A. pictum</i> 'Tricolor'	–	–	–	–	–	–	–

istic of *A. rotundum*. Cluster VI cultivars, like *A. modestum*, are either solid green or slightly variegated. Foliar variegation in cluster VII cultivars generally has rather ashy to silvery irregular blotches that are close to the pattern of *A. pictum*.

Five other genera of Araceae (*Alocasia*, *Anthurium*, *Dieffenbachia*, *Philodendron* and *Spathiphyllum*) were included in this study. The five genera were properly positioned outside the *Aglaonema* cluster. They share genetic similarity no greater than 0.10 based on the Jaccard's coefficient with any *Aglaonema* samples and the genetic similarities among these five genera are < 0.14.

#### Genetic relationships among species

Seven of the 54 cultivars are not of hybrid origin: *A. commutatum* 'Echo' and 'Trebuii', *A. crispum* 'B.J. Freeman', *A. nitidum* 'Curtisii' and 'Ernesto's Favorite', *A. modestum* and *A. pictum* 'Tricolor'. Other species that were indirectly involved in this study include *A. brevispathum*, *A. costatum*, *A. marantifolium* and *A. rotundum*; they were parents of the hybrids used in the study. *Aglaonema pictum* and *A. modestum* are rather dissimilar since their Jaccard's similarity coefficient is only 0.31, and both are distant from the other species (Table 3). The distant relationships between these two species and from the others may be attributed to their originating geographical locations. *Aglaonema pictum* is native to Sumatra and inhabits the slopes of major volcanoes between 1000 and 2000 m a.s.l (Nicolson, 1969), which may isolate it from the other species. The origin of *A. modestum* in southern China, northern Laos and northern Thailand is certainly separate from the others, which are generally native to Malaysia, Indonesia and the Philippines. Hybrid cultivars related to *A. marantifolium* and *A. rotundum* are situated in clusters III, IV and V; Jaccard's similarity coefficients across the three clusters range from 0.61 to 0.68. *Aglaonema marantifolium* originates from Moluccas through New Guinea, whereas *A. rotundum* is native to Sumatra (Jervis, 1980). However, genetic similarity manifested by their hybrids may imply that the two species are relatively close. *Aglaonema costatum* and *A. brevispathum* are native to continental south-east Asia and were considered to be morphologically similar by Nicolson (1969). When both were crossed with *A.*

*commutatum*, their hybrids, 'Emerald Star' and 'Stars', resemble each other and share a Jaccard's similarity coefficient of 0.82. Cultivars related to *A. crispum*, *A. nitidum* and *A. commutatum* are mainly gathered in cluster I, and most of them have a Jaccard's similarity coefficient above 0.80. *Aglaonema commutatum* originates from the Philippines and north-eastern Celebes (Nicolson, 1969). Both Nicolson (1969) and Jervis (1980) believed that *A. commutatum* could actually be hybrid in origin because chromosome counts of *A. commutatum* cultivars are polyploid ( $2n = 120$ ) (Jones, 1957; Nicolson, 1969). *Aglaonema crispum* is native to Mount Bulusan, Sorsogon Province, and Luzon, Philippines with a chromosome number of  $2n = 60$  (Nicolson, 1969). *Aglaonema nitidum* is more widely distributed from Southern Burma, Malaysia and Sumatra to north Borneo and possibly north-eastern Celebes. However, there is no documented information about the chromosome number of *A. nitidum*. The locations where *A. crispum*, *A. nitidum* and *A. commutatum* are all in a close proximity, and the close genetic similarity among the three species (Table 3) and their related cultivars in cluster 1, lead us to believe that *A. crispum* and *A. nitidum* are probably progenitors of *A. commutatum*. Further studies with more species combined with cytological analyses are warranted to test the origin of *A. commutatum*.

#### Implications for *Aglaonema* breeding

Clear separation of all 54 *Aglaonema* cultivars proves the sensitivity of AFLP markers for cultivar identification. This sensitivity is further illustrated by the fact that two cultivars selected from the same cross can be distinguished. For example, 'J.G.' and '1502' were  $F_1$  progenies of the same cross between *A. commutatum* 'Tricolor' and  $A. \times$  'Manila'. Due to their parental heterozygosities,  $F_1$  is actually a segregating generation. Selection can thus be initiated in the  $F_1$  generation, and desirable individuals can be reproduced by vegetative propagation. The AFLP analysis can also detect differences between somaclonal variants. Both 'Moonlight Bay' and 'Diamond Bay' are somaclonal variants selected from 'Silver Bay'; Jaccard's similarity coefficient between 'Silver Bay' and the two variants was 0.79. In addition to proving the discriminatory power of AFLP analysis, this separation may suggest that some somaclonal variants are actually significantly different



genetically. Genetic differences between somaclonal variants and their parents have also been detected in *Arabidopsis thaliana* (L.) Heynh. (Polanco and Ruiz, 2002), *Carya illinoensis* (Wangenh.) C. Koch (Vendrame et al., 2000), *Dieffenbachia* (Chen et al., 2003) and *Quercus suber* L. (Hornero et al., 2001) by AFLP analysis. Additionally, it was possible to identify unique fragments for four cultivars: one fragment for 'Treibii', two fragments for '714-12', ten for *A. pictum* 'Tricolor' and four for 'XIV'.

Furthermore, cultivars with some similar physiological traits are also clustered. For example, one of the most important problems in *Aglaonema* production and interior use is chilling injury. 'Emerald Star' and 'Stars', two of the most chilling-resistant cultivars (Chen et al., 2001), are clustered together in the dendrogram. Basal shoot formation is also an important trait for *Aglaonema* because a cultivar with more basal shoots will need fewer cuttings per container to produce a full appearance in the final product. Basal shoots can also be removed and used as propagules since *Aglaonema* is propagated mainly by shoot cuttings or division. Cultivars '277028', 'Emerald Bay', 'Golden Bay', 'Moonlight Bay' and '257-12', all clustered together in the dendrogram, produce multiple shoots, requiring fewer cuttings per container in production. The correlation between AFLP markers and these desirable traits will help future selection of cultivars that are resistant to chilling or that produce multiple shoots.

However, with most hybrid cultivars gathered in cluster I with Jaccard's similarity coefficients of more than 0.84, there must be a serious concern over the potential genetic vulnerability of *Aglaonema* cultivars to either diseases or pests because these hybrids are among the most popular and most commonly grown cultivars in production. This finding is unexpected because many of them are interspecific hybrids, and presumed to be genetically diverse. The AFLP analysis suggests that due to the natural hybridization among some species, parental materials used in interspecific hybridization are actually genetically rather similar. To avoid genetic vulnerability, parental materials with wide genetic diversity should be considered for future hybrid development.

As far as is known, this is the first molecular investigation of the genetic relationships of *Aglaonema* species and cultivars. It was possible to identify 54 *Aglaonema* cultivars based on polymorphism generated by six primer sets. The AFLP profiles and the clusters identified can be used as a basis for comparison of other *Aglaonema* species or cultivars. The genetic similarity among cultivars established can help future *Aglaonema* germplasm identification, conservation and new cultivar development.

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