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To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

3. Domir, S., L.R. Schreiber, and J.M. Ichida. Factors affecting growth of *Ophiostoma ulmi* on elm callus tissue. J. Environ. Hort. 9:211–215.

4. Helgeson, J.P. 1983. Studies of host-pathogen interactions in vitro. *In*: Use of Tissue Culture and Protoplasts in Plant Pathology. J.P. Hegeson and B.J. Deverall (Eds.). Academic Press, New York, NY pp. 9–38.

5. Jacobi, W.R., H.V. Amerson, and R.L. Mott. 1982. Microscopy of cultured loblolly pine seedlings and callus inoculated with *Cronartium fusiform*. Phytopathology 72:138–143.

6. Jang, J.C. and F.H. Tainter. 1990. Cellular responses of pine callus to infection by *Phytophthora cinnamomi*. Phytopathology 80:1347–1352.

7. Krause, C.R. and C.L. Wilson. 1972. Fine structure of *Ceratocystis ulmi* in elm wood. Phytopathology 62:1253–1256.

8. MacDonald, W.L. and H.S. McNabb. 1974. Electron microscope observations of *Ceratocystis ulmi*-induced tylosis development in *Ulmus*. Eur. J. For. Pathol. 4:2–10.

9. Miller, S.A. and D.P. Maxwell. 1983. Evaluation of Disease Resistance. *In*: Handbook of Plant Cell Culture. Vol. 1. Techniques for Propagation and Breeding. D.A. Evans, W.R. Sharp, P.V. Ammirato, and Y.Y. Yamada (Eds.). Macmillan, New York. pp. 853–879.

10. Ouellette, G.B. 1978. Fine structural observations on substances attributable to *Ceratocystis ulmi* in American elm and aspects of host cell disturbances. Can. J. Bot. 56:2550–2566.

11. Ouellette, G.B. 1978. Ultrastructural observations on pit membrane alterations and associated effects in elm xylem tissues infected by *Ceratocystis ulmi*. Can. J. Bot. 56:2567–2588.

12. Ouellette, G.B. 1978. Light and electron microscope studies on cell wall breakdown in American elm xylem tissues infected with Dutch elm disease. Can. J. Bot. 56:2666–2693.

13. Pijut, P.M., R.D. Lineberger, S.C. Domir, J.M. Ichida, and C.R. Krause. 1990. Ultrastructure of cell of *Ulmus americana* cultured in vitro and exposed to culture filtrate of *Ceratocystis ulmi*. Phytopathology 80:764–767.

14. Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17:208–212.

15. Went, J.C. 1954. The Dutch elm disease-summary of fifteen years hybridization and selection work (1937–1952). Tijdschr. PlZiekt 60:109–127.

Identification of Genetic Diversity among *Loropetalum chinense* var. *rubrum* Introductions[1]

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Abstract

Loropetalum chinense (R.Br.) Oliv. is an Asiatic evergreen shrub with many desirable landscape characteristics. It is fast-growing, tolerant to disease and insects, and produces an abundance of attractive flowers. Recently, the horticultural community has shown an increased interest in the species since several pink-flowering selections *(L. chinense* var. *rubrum)* have become available. Due to the relatively rapid introduction and commercialization of *Loropetalum chinense* var. *rubrum*, there has been some confusion concerning the identity and distinctness of cultivars. This research used Randomly Amplified Polymorphic DNA (RAPDs) to examine the genetic diversity of 14 *L. chinense* var. *rubrum* accessions, and 2 accessions of *L. chinense*. Results indicate the presence of 4 groupings among the introductions, with many of the introductions having at least 1 or 2 other closely related selections. Most named introductions were closely related to other unnamed introductions.

Index words: RAPD, DNA fingerprinting, breeding.

Species used in this study: *Loropetalum chinense* (R.Br.) Oliv., *Loropetalum chinense* var. *rubrum* Yieh.

Significance to the Nursery Industry

Many *Loropetalum chinense* var. *rubrum* introductions have been established into North America since the 1980s. Many of these selections have been introduced by different individuals; however, plant material has been obtained from the same sources. This circumstance may lead to different cultivars being named from the same material. This research uses DNA markers to examine *L. chinense* var. *rubrum* introductions to identify diversity among introductions. The results indicate the presence of 4 groupings among the introductions. Many of the introductions, however, have at least 1 or 2 other closely related selections. This information will provide growers with a basis for decision-making to avoid redundancy in cultivar propagation. Also, the data presented are useful when making breeding decisions concerning *L. chinense* var. *rubrum.*

Introduction

Loropetalum chinense (R.Br.) Oliv. is an Asiatic evergreen shrub that belongs to the witchhazel family. Its natural range is in the Chinese provinces from Shantung to Yunnan, and in a small area of Japan (1). Traditionally, *Loropetalum* has been used for medicinal purposes by the Chinese (2, 3), and for charcoal by the Japanese (1).

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Table 1. Source and characteristics of Loropetalum chinense taxa examined.

Accession (USNA Code)	Year brought to North America	Leaf/ flower color	Asiatic source	Route	Cultivar name
A (160)	1989	green/pink	Nihon Kaki Nursery, Japan	John Creech → Sylvester March U.S. National Arboretum	'Blush'
B (176)	1990	green/pink	Nihon Kaki Nursery, Japan	Ozzie Johnson → Atlanta Botanic Garden → J.C. Raulston	—
C (274)	1991	green/pink	Kairyo En Nursery, Japan	Barry Yinger → Hines Nurseries, TX	—
D (275)	1989	green/pink	Watanabe Nursery, Japan	Audry Teasdale (Monrovia Nurseries, CA)	Razzleberri™
E (161)	1989	red/pink	Nihon Kaki Nursery, Japan	John Creech → Sylvester March U.S. Nat. Arboretum	'Burgundy'
F (162)	1989	red/pink	Shanghai Botanic Garden, China	James Waddick → J.C. Raulston	'Burgundy'
G (173)	1989	red/pink	Shanghai Botanic Garden, China	James Waddick → Arnold Arboretum → Mike Dirr (University of Georgia)	'Burgundy'
H (273)	1991	red/pink	Shun So En Nursery, Japan	Barry Yinger → Hines Nurseries, TX	Plum Delight™
I (282)	1991	red/pink	Nihon Kaki Nursery Japan	Mark Krautmann (Heritage Seedlings) → Gayle Suttle (Microplant Nurseries)	Sizzlin' Pink
J (323)	1989	red/pink	Watanabe Nursery, Japan	Audrey Teasdale (Monrovia Nurseries)	—
K (324)	1989	red/pink	Nanjing Botanic Garden, China	Jian Gsu Pipa Horticultural Co. (China) → P. Piroche (Piroche Nurseries, B.C.)	—
L (326)	1987	red/pink	Nanjing Botanic Garden, China	Jian Gsu Pipa Horticultural Co. (China) → P. Piroche (Piroche Nurseries, B.C.)	Fire Dance™
M (888)	1991	red/pink	Kairyo En Nursery, Japan	Barry Yinger	—
N (999)	1994	red/pink	Shibamichia Honten Nursery, Japan	Sylvester March (USNA)	—
O (174)	Unknown	green/white	Unknown	Magnolia Nursery, AL	—
P (325)	1987	green/white	Nanjing Botanic Garden, China	Jian Gsu Pipa Horticultural Co. → P. Piroche (Piroche Nurseries, B.C.)	Snow Dance™

Loropetalum was first introduced to North America as a landscape plant in the early part of this century (4). In the US, growth of *Loropetalum* is best suited to USDA hardiness zones 7b to 9 (5, 6). Despite its early introduction and favorable landscape qualities (abundant flowers, tolerance to disease and insects), *Loropetalum* is underutilized in southern landscapes (4, 6).

The availability of pink-flowering selections of *Loropetalum* (*Loropetalum chinense* var. *rubrum* Yieh) has stimulated interest in the species among the horticultural community (7, 8). Over 15 introductions have been established in North America since the late 1980s. Since many of these introductions were brought to North America by different individuals, but collected from the same sources, several of the introductions are possibly the same. The independent naming of these introductions has already caused confusion in the woody ornamental industry.

The introductions are difficult to identify by morphological characteristics. Although differences in leaf size and coloration are evident, stage of growth, cultural conditions, and environment influence uniformity. Additionally, it is difficult to distinguish unique cultivars because none of the introductions have been grown to maturity in North America, and few have been compared side by side.

This work determines the distinctiveness of the *L. chinense* var. *rubrum* introductions by examining DNA similarities using Randomly Amplified Polymorphic DNA (RAPD) markers (9, 10). When used to examine woody landscape plants, RAPD markers will not define cultivars because a cultivar may consist of more than one clone. However, this procedure does allow for the identification of clones, and may provide insight into which clones are closely related and which are genetically distinct.

Materials and Methods

Fourteen pink-flowering and 2 white-flowering clones were assembled by the U.S. National Arboretum (Table 1)

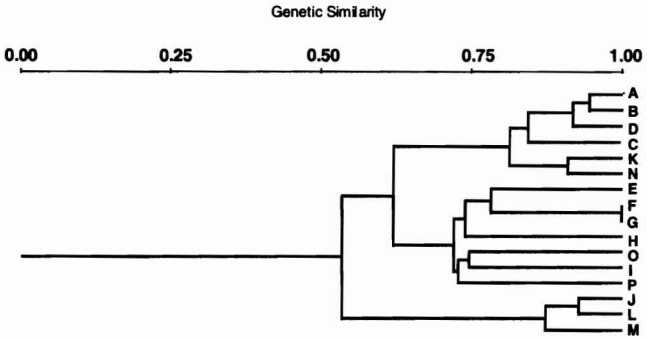

Fig. 1. Cluster analysis of RAPD data using the unweighted pair-group method, arithmetic average (UPGMA) method. Taxa codes are as per Table 1.

and maintained under greenhouse conditions. The 14 pink-flowered accessions represented the breadth of diversity in *L. chinense* var. *rubrum* in North America as of August 1994. Since then, four other taxa have been reported by Dirr et al. (11) and a fifth is being evaluated by Hines Nurseries (Robert Grove, pers. comm.).

Fully expanded leaves were removed from plants and frozen at –20C (–5F) until used. DNA was extracted from approximately 0.5 g of leaf tissue using the nuclei-concentration method of Wilson (12), followed by the CTAB-based DNA isolation technique of Saghai-Maroof et al. (13).

RAPD reactions were performed as per Gawel and Bartlett (14). Thirty RAPD primers were used: OPA1–20 and OPB1–10 (Operon Technologies, Alameda, CA). Products were separated on 2% TAE agarose gels (15), stained with ethidium bromide and photographed. Size and brightness of amplified products were determined using RFLPScan (CSPI, Billerica MA). Only products that were between 250 and 1,000 bp and in the upper 75 percentile of brightness were included in the analysis. RAPD products that differed in size by more than 1% were considered different. Genetic distance calculations (16), principal components analysis (PCA) and UPGMA clustering were computed using NTSYS-pc (17).

Results and Discussion

The nuclei concentration step in the DNA extraction procedure was a prerequisite to obtain amplifiable DNA from *L. chinense* var. *rubrum*. When this step was not included, either no DNA, or DNA that would not amplify was obtained. The described method was designed to separate nuclei from cytoplasmic components (12). Tissues of *L. chinense* are known to contain high levels of tannins (3, 18), and it is surmised that these compounds were responsible for contaminating the DNA when the nuclei concentration step was not used.

Of the 30 primers used, 18 produced monomorphic results and 12 produced polymorphic results. A total of 57 polymorphisms were scored from these primers. The statistical analyses included only data from the primers that yielded polymorphisms. Results of the UPGMA analysis and PCA are presented in Figures 1 and 2, respectively. The UPGMA analysis depicts 4 groupings of accessions. While there are similarities within each group for leaf and/or flower color, these groupings do not correlate exclusively with leaf or

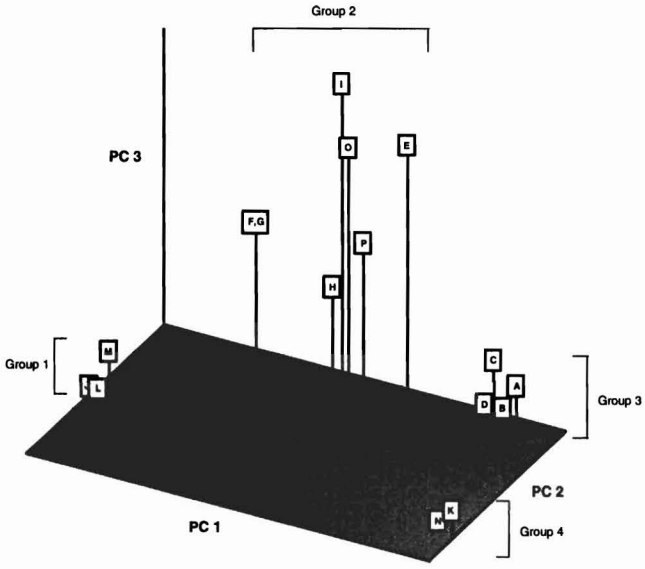

Fig. 2. Principal Components Analysis (PCA) of RAPD data. Taxa codes are as per Table 1.

flower color. The same circumstance is observed in the principal components analysis (Fig. 2). For example, accession I (Sizzlin' Pink) has red leaves and pink flowers, yet according to the RAPD data, its DNA was most similar to accessions O and P (green leaves, white flowers).

The principal components analysis presents a more graphical representation of the data than does the UPGMA clustering. This treatment of the data illustrates the closeness of the relationships within most of the groups, and illustrates the diversity in the centermost group of the UPGMA analysis. Accessions F and G showed no differences in RAPD banding patterns. These accessions came to the USNA by different routes, but the cuttings were taken from the same plant at the Shanghai Botanic Garden (Table 1).

The four green-leafed, pink-flowering accessions (A, B, C, D) grouped together and exhibited similar morphological characteristics when compared as small plants under greenhouse conditions at the USNA (R. Johnson, unpublished). Three of the four are considered to be the same cultivar (A, B and C). Dirr et al. (11) evaluated selected varieties of *L. chinense* var. *rubrum* based on morphological characteristics, and concluded the varieties 'Blush' (A) and 'Razzelberri' (D) are probably clones. Our analysis revealed a close, but not identical, relationship between these clones. Five of the 57 RAPD fragments were different. This contradiction may be due to the relatively recent introduction of *L. chinense* var. *rubrum*. Little information exists concerning the behavior of varieties as they mature, and most morphological comparisons to date have been performed on immature specimens. Although all four accessions (A, B, C, D) looked similar at a young age, differences may become apparent in mature specimens. Conversely, if genetically distinct clones are morphologically indistinguishable, the clones may be considered the same cultivar.

The most diverse group appears to be Group 2 (Fig. 2). Even though two different clones of the red-leafed types are similar enough that they have been named 'Burgundy' (E, and F/G), they are relatively distinct based on RAPD data. Accession I was named 'Burgundy', but has been re-named

Sizzlin' Pink. Accessions E and I both came from the same nursery, but via different routes and in different years. Although our analysis places them in the same general group, they are separated within this group.

Dirr et al. (11) reports sufficient morphological differences in L (Fire Dance™) and 'H' (Plum Delight™) to warrant defining these accessions as different cultivars. Our analysis also makes this distinction. Our analysis found accessions K and N to be similar to each other, and distinct from the other groupings. A similar circumstance is found in the relationships between accessions L (Fire Dance™), J and M.

The data presented here indicate RAPD differences among plants with similar leaf and flower colors. It also indicates that a large proportion of these plants have at least one other similar accession. Knowledge of these relationships is important when making breeding decisions concerning *L. chinense* var. *rubrum*. For example, if the production of a genetically diverse population is desired, within-group hybridizations of plants from groups 1, 3 or 4 would not produce as much variability as between-group crosses of these plants. The data presented is also useful to growers who want to reduce redundancy in the varieties of *L. chinense* var. *rubrum* they choose to propagate. Additionally, for those interested in the establishment of collections of *L. chinense*, the information presented here will be useful in determining which accessions will contribute the most or least genetic diversity to a collection.

Literature Cited

1. Creech, J.L. 1960. On the distribution of *Loropetalum chinense*. Amer. Hort. Mag. 39:236.

2. Chiang Su New Medicinal College. 1977. Dictionary of Traditional Chinese Crude Drugs. Shanghai Scientific Technologies, Shanghai.

3. Yoshida, T., O. Namba, L. Chen, Y.Z. Liu, and T Okuda. 1990. Ellagitannin monomers and oligomers from *Euphorbia prostrat* Ait. and oligomers from *Loropetalum chinense* Oliv. Chemical and Pharmaceutical Bulletin 38: 3296–3302.

4. Creech, J.L. 1986. Colorful oriental plants can dress up US gardens. Amer. Nurseryman 163:54.

5. United States Department of Agriculture/Agricultural Research Service. 1990. USDA plant hardiness zone map. Miscellaneous publication 1475, US Government Printing Office, Washington, DC.

6. Dirr, M.A. 1990. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation, and Uses. 4th edition. Stipes Publishing Co., Champaign, IL.

7. Johnson, R. 1993. As I see it: a tale of two plants. Amer. Nurseryman 178(6): 7, 12–13.

8. Dirr, M.A. 1993. *Loropetalum chinense* var. *rubrum*: As a specimen in a grouping in masses, or in a container, the shrub has excellent potential for the landscape. Nursery Manager 9(1):24–26.

9. Welsh, J., C. Peterson, and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 19:7213–7218.

10. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531–6535.

11. Dirr, M.A., Y.Q. Huo, and S.A. Hartzler. 1995. A synopsis of pink-flowered *Loropetalums*, *Loropetalum chinense* var. *rubrum*. Nursery Management and Production 11:30–31.

12. Wilson, H.D., J. Doebley, and M. Duvall. 1992. Chloroplast DNA diversity among wild and cultivated members of Cucurbita (Cucurbitacea). Theor. Appl. Genet. 84:859–865.

13. Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Nat. Acad. Sci. USA 81:8014–8018.

14. Gawel, N.J. and A.C. Bartlett. 1993. Characterization of differences between whiteflies using RAPD-PCR. Insect Molecular Biology 2(1):33–38.

15. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. 2nd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

16. Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull Sco. Vaud. Sci. Nat. 44:223–270.

17. Rohlf, F.J. 1992. NTSYS-pc numerical taxonomy and multivariate analysis system. Ver 1.70. Exeter Software, Setauket, NY.

18. Yoshida, T., S. Tanei, Y.Z. Liu, K. Yuan, C.R. Ji, and T. Okuda. 1993. Hydrolsable tannins from Loropetalum chinense. Phytochemistry 32: 1287–1292.