Using Molecular Markers to Investigate Parentage of Azaleodendron Hybrids

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Significance to Industry: Plant breeding programs benefit greatly from a thorough understanding of characteristics and genetics of parental germplasm. Unfortunately, many ornamental cultivars are of unknown origin and parentage, raising questions regarding their nomenclature, genetics, and utility in breeding programs. Historically, unknown hybrids were identified using taxonomically relevant characteristics such as floral morphology. This can be an effective means of identification but it can also lead to confusion when dealing with closely allied taxa. Advancements such as the polymerase chain reaction (PCR) and amplified fragment length polymorphisms (AFLP) have provided researchers with the tools for stringent DNA analysis through the use of molecular markers. Using these techniques it is now possible to screen putative parents against the hybrid of interest to quantitatively identify the parents. This technique was successfully used to determine that *Rhododendron* 'Fragrant Affinity' and 'Fragrans' are distinct cultivars of similar parentage. Additionally, AFLP markers revealed that *R. catawbiense* L. is not one of the parents as has been proposed, but is more likely a hybrid of *R. ponticum* L.

Nature of Work: A number of inter-subgeneric hybrids between evergreen rhododendrons and azaleas have been reported and are frequently referred to as azaleodendrons. The history of *R.* 'Fragrant Affinity' is vague. The name 'Fragrant Affinity' is not registered and we have been unable to find documentation on its origin, but has been reported to be a hybrid of *R. catawbiense* x *R. viscosum* (L.) Torr. (Kehr, personal communication). *R.* 'Fragrant Affinity' is similar in appearance to another azaleodendron, *R.* 'Fragrans'. *Rhododendron* 'Fragrans', also reported to be a hybrid of *R. catawbiense* x *R. viscosum* was introduced by Paxton of Chandler & Sons Nursery, London, in 1843, and is described as, "A sweet-scented azaleodendron, fast-growing and compact with trusses of small flowers, pale mauve with centers lighter to white," (6).

The purported parents of *R.* 'Fragrant Affinity' are taxonomically distinct. *R. catawbiense* is in the subgenus *Hymenanthes*, section *Ponticum*, subsection *Pontica* (3). This subsection contains evergreen species from North America, Europe, and Asia, including *R. hyperythrum* L. *Rhododendron viscosum* is in the subgenus *Pentanthera*, section *Pentanthera*, subsection *Pentanthera*. This subsection contains other fragrant, deciduous species from North America including *R. arborescens* (Pursh) Torrey, *R. atlanticum* (Ashe) Rehd. and *R. canescens* (Michx.) Sweet.

Molecular techniques can be used to assess genetic relationships among plants. The use of polymorphisms produced by arbitrarily primed polymerase chain reaction (AP-PCR) can distinguish between species as well as cultivars of the same species (2, 5, 7). AFLPs are PCR based markers used in the rapid detection of genetic diversity. The objectives of this project were to use these molecular techniques to identify the progenitor species of *Rhododendron* 'Fragrant Affinity' and determine if *R.* 'Fragrant Affinity' and *R.* 'Fragrans' are synonyms or distinct clones.

Materials and Methods: *Plant Material*: In order to elucidate the progenitor species of *R.* 'Fragrant Affinity' we observed DNA polymorphisms in the hybrid that are present in each parental species yet distinct from closely related species. To accomplish this we included clones of the purported parents *R. catawbiense* and *R. viscosum* as well as related taxa. We compared *R. arborescens*, *R. atlanticum*, and *R. canescens* from subsection *Pentanthera* as well as *R. hyperythrum*, *R. ponticum*, and *R. maximum* L. from subsection *Pontica*. *Rhododendron* 'Fragrans' was also evaluated to compare to 'Fragrant Affinity' and putative parents. All material was maintained at The Mountain Horticultural Crops Research and Extension Center in Fletcher, NC or J.C. Raulston Arboretum in Raleigh, N.C. except for *R.* 'Fragrans' which was provided by Harold Greer, Eugene, Ore.

DNA extraction: A CTAB (Cetyltrimethylammonium bromide) extraction method described by Affandor et al. (1), modified using the Fast Prep FP120 (Thermo Savant, Holbrook, N.Y.) to grind tissue was used for isolation of nuclear DNA. Approximately 100-150 mg of tissue from newly opening leaves was collected in 2.0 ml conical tubes and kept cold until extraction.

DNA amplification and electrophoresis: DNA amplification was performed using six primer combinations under conditions described by Milla et al. (4). All primers and adapters were obtained from Sigma Genosys (The Woodlands, Texas) with the exception of labeled primers, which were obtained from LICOR Inc. (Lincoln, Neb.). Amplification products were separated on a 0.8% polyacrylamide gel for 3-hours in a Licor IR2 two-dye DNA sequencer using a 50-700bp standard

Data Analysis: AFLP-Quantar 1.0 (Keygene Products B.V., Wageningen, Netherlands) software package was used to score distinct, major, reproducible bands. Presence or absence of each AFLP fragment was scored as a binary unit character (present = 1, absent = 0). The simqual function in NT SYSpc 2.1 (Exeter Software, Setauket, New York) was used to calculate Jaccard's similarity coefficients and dendrograms were created using the unweighted pair group method with arithmetic averages (UPGMA).

Results and Discussion: The six primer combinations selected generated extensive DNA polymorphisms. We scored 139 bands ranging in size from 75 to 575 bp. The level of variation was very great between species and cultivars suggesting that with further analysis it may be possible to develop cultivar and species specific profiles. All samples were repeated at least twice except *R. hyperythrum* 1 and *R. viscosum* 7 and the degree of reproducibility was high as exhibited by the nearly identical band patterns.

Based on binary band-share data, Jaccard's coefficients of relationships were calculated and used to generate pairwise relationships (data not shown) and a dendrogram showing relationships among taxa (Figure 1). Using banding patterns and calculated genetic similarity it was clear that *R. catawbiense* is not a parent of 'Fragrant Affinity' or 'Fragrans'. The two cultivars were nearly 80% genetically similar suggesting that they likely share the same parentage. Due to numerous monomorphic bands among species in the subsection *Pentanthera* it was not possible to determine the exact deciduous *Rhododendron* parent but there was a band at ~475 bp which was only present in one population of *R. viscosum* and *R.* 'Fragrant Affinity'. The high level of polymorphism observed between groups shows that there is potential to use more primer combinations to resolve this group.

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Figure 1. Dendrogram showing grouping of related taxa by genetic similarity based on data derived from AFLP analysis (calculated using Jaccard's coefficient of similarity).