A Comparison of Suspected “Maggie” Roses Utilizing Randomly Amplified Polymorphic (RAPD) DNA Analysis

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Acknowledgments. The authors are indebted to Dr. Malcolm Manners of Florida Southern College for his assistance during every step of this project, and to Liesbeth Cooper, Peggy Martin, and Erich Unmuth for the plant samples.

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Materials and Methods

PLANT MATERIAL. Frozen samples of leaves from roses grown under the name “Kakinada Red” and another under the name of “Maggie” were a generous gift of Peggy Rose Martin (Gonzales, LA). Samples of leaves of “Pacific” were obtained from Blue Meadows Nursery, Bermuda (generous gift of Liesbeth Cooper). Another sample of “Maggie,” as well as Rosa laevigata (‘Cherokee’ rose) were grown on the campus of Florida Southern College. Once obtained, all samples were stored at –20 °C until used.

DNA ISOLATION. Genomic DNA was isolated utilizing a DNeasy Plant Mini kit (QIAGEN, Valencia, CA) based on the manufacture’s protocol with some modifications. For plant homogenization, 0.1 g of leaf material was quick frozen in a dry ice/ethanol bath and then ground in a chilled mortar and pestle before extraction. After extraction, two phenol (1:1 v:v) extractions and one chloroform (1:1 v:v) extraction were carried out. To precipitate DNA, one 10th volume of 3 M sodium acetate and 2.5 volumes of absolute ethanol were added to the sample. The sample was incubated on ice for 30 min and then centrifuged for 20 min at 4 °C at 14,000 rpm. The pellet was air dried for approximately 15 min and re-suspended in 100 µL of TE buffer. A 0.5% agarose gel was used to confirm DNA isolation.
**PCR CONDITIONS.** RAPD PCR analysis of the samples was carried out utilizing GoTaq® PCR Core System II (Promega Corporation, Madison, WI) according to the manufacturer’s protocol. Individual reactions were carried out with one of five primers: OPA-05: 5’ AGGGGTCTTG 3’; OPA-08: 5’ GTGACGTAGG 3’; OPA-09: 5’ GGGTAACGCC 3’; OPC-05: 5’ GATGACC-GCC 3’; or OPC-09: 5’ CTCACCGTCC 3’ (Sigma-Aldrich, St. Louis, MO). Each PCR reaction contained 1× Taq buffer, 2.5 mM MgCl$_2$, 200 µM each of dATP, dTTP, dCTP, dGTP, 10 pmol of one primer, 1 unit Taq polymerase, and 10 ng genomic DNA, in a final volume of 50 µL. Each sample was overlaid with 50 µL mineral oil. PCR amplification was carried out as follows: 5 min at 94 °C, then 41 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C, and then held at 4 °C. A 20-µL portion of each PCR reaction was run on a 1.5% agarose gel, stained with 0.5 µg/mL ethidium bromide and viewed on an ultraviolet illuminator. The gels were photographed and analyzed. Each isolation and RAPD PCR reaction was performed at least three times with each rose variety and each primer.

**Results and Discussion**

**Primer banding patterns.** To test the ability of the different primers to show variations in banding patterns upon amplification of the DNA, the control rose, *Rosa laevagata*, was used in RAPD PCR with the five different primers. As shown in Fig. 1, all primers produced a different banding pattern with the same *R. laevagata* template DNA, indicating each primer successfully targets and amplifies different regions of the DNA.

**Analysis of suspected “Maggie” roses.** The “Maggie” sample grown on the campus of Florida Southern College, the “Maggie” supplied by P. Martin in Louisiana, and the acquired samples of “Kakinada Red” and “Pacific” were compared with each other and the unrelated *R. laevagata*. Each sample was amplified using all five primers, each in a separate reaction. Two representative gels are shown in Fig. 2 using primers OPA-05 (Fig. 2A) and OPA-08 (Fig. 2B). The banding patterns of the “Maggie” samples, “Kakinada Red,” and “Pacific” appear to be identical (and distinct from *R. laevagata*), indicating all are the same rose. RAPD PCR does not show small variations within the DNA, so these varieties may have some genetic differences among them. However, any possible mutations did not result in detectable differences in the RAPD profiles.

Based on the histories of these roses and the genetic data presented here, the rose found by William Welch, which he named “Maggie,” is not a unique hybrid. If the histories are accurate, “Pacific” was introduced to Bermuda over 150 years ago via the captain of a French ship, and “Kakinada Red” has been growing in India for several centuries (Lowery, 2006). It is possible that the rose we call “Maggie” was originally from India, transported to Bermuda, and then brought to the U.S. Or “Maggie” may have come directly from India. This scenario precludes the possibility that “Maggie” is the same rose as ‘Eugenie E. Marlitt’, and nullifies the claims by many breeders and nurseries that they are the same rose originally bred by Geschwind.

Samples of ‘Eugenie E. Marlitt’, ‘Julius Fabianics de Misefa’, and another “Maggie” were donated to our lab by Erich Unmuth (Vienna, Austria). However, we were unsuccessful in isolating DNA from these samples for RAPD PCR. For a full analysis, and to provide a more complete picture of the origin of the “Maggie” roses, further work comparing the “Maggie” samples with these other varieties is essential.
Literature Cited