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

Ashley Wilson and Nancy Morvillo*

Department of Biology, Florida Southern College, 111 Lake Hollingsworth Drive, Lakeland, FL 33801

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Previous analysis using RAPD PCR has been helpful in determining the genetic relationships of different varieties of roses and elucidating the parentage of hybrids. We utilized the same approach to answer questions regarding the identity of several varieties of roses thought to be similar or identical to each other: "Maggie," "Kakinada Red," "Pacific," and 'Eugenie E. Marlitt'. These roses grow well in many different regions. "Maggie" is a fragrant burgundy-red rose found by William Welch in Louisiana in 1980. "Kakinada Red" is found in India, while "Pacific" comes from Bermuda. It has been hypothesized that these roses may actually be Rudolf Geschwind's hybrid 'Eugenie E. Marlitt'. While these varieties appear to be similar, historical accounts cannot confirm if they are the same rose. We used RAPD PCR to investigate their identities. Based on this analysis, at least some of these varieties appear to be identical.

Confirmation of the identity of some historical roses has been problematic, due to inaccurate or lost records. One variety that fits this category is "Maggie," a found rose discovered by William Welch in Louisiana (Lowery, 2006; Unmuth, 2006; Welch, 1990). Welch noted the apparent similarity of "Maggie" to roses grown in Texas (Welch, 1990). "Maggie" grows well in different environments, from full sun to mostly shade, with variations in thorns (Lowery, 2006), and it can be trained as a climber (Welch, 2006). Welch concluded it must have China Rose heritage, based on its blooming properties and its ease of propagation, and he classified it as a Bourbon (Welch, 1990).

The source of "Maggie" is unclear, but there has been speculation on its origin. 'Eugenie E. Marlitt' (of which multiple spelling variations exist), a rose propagated by Rudolf Geschwind at the end of the 19th century, was a very popular variety in America in the early 20th century (Beales, 1992). It appears to be the same as "Maggie" (Lowery, 2006; Unmuth, 2006). Another rose, 'Julius Fabianics de Misefa', may be identical to 'Eugenie E. Marlitt' (Unmuth, 2006). However, the records of 'Eugenie E. Marlitt's distribution in the U.S. and elsewhere are sketchy and incomplete, making it difficult to definitively uncover its lineage and its possible relationship to other roses (Lowery, 2006). In addition, Geschwind sold unnamed seedlings to nurseries in Europe and the U.S., so many roses related to 'Eugenie E. Marlitt' could have spread far and wide (Unmuth, 2006).

Adding to the problem of the identity of "Maggie" are some clues that point to a possibly older origin of this rose. It appears identical to "Pacific," a rose grown in Bermuda that is fabled to have been brought to the island in the early 19th century, which would make it unrelated to 'Eugenie E. Marlitt' (Lowery, 2006). And "Maggie" shows striking similarity to a rose grown in India as "Kakinada Red" (Lowery, 2006). So, while these varieties appear similar, their sporadic histories do not allow us to conclusively resolve whether or not they are the same rose.

In this study, RAPD PCR, a procedure that randomly copies regions of DNA, was used. This type of analysis has allowed for the comparison of genomes of multiple types of plants (for some recent examples, see Ebrahimi et al., 2012; Gupta et al., 2012; Schlag and McIntosh, 2013), and has been successful in solving several rose mysteries (Frederick et al., 2002; Lewis et al., 2004; Manners et al., 2004; Morvillo, 2004; Wagner et al., 2002; Walker and Werner, 1997). Based on the analysis presented here, all of the tested samples of "Maggie," "Kakinada Red," and "Pacific" appear to be identical.

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 laevagata* ('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 resuspended in 100 µL of TE buffer. A 0.5% agarose gel was used to confirm DNA isolation.

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*Corresponding author; phone: (863) 680-6240; email: nmorvillo@flsouthern.edu

PCR conditions. RAPD PRC 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' GATGACCGCC 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.

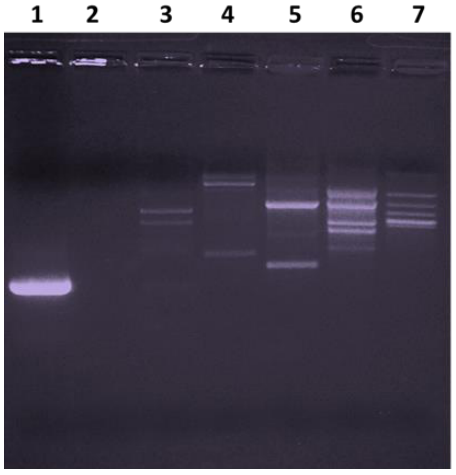

Fig. 1. RAPD analysis of *Rosa laevagata* with different primers. DNA was isolated from *R. laevagata* and subjected to RAPD analysis as described in Materials and Methods. A photograph of the resulting gel is shown. Lane 1: positive control reaction from the Promega GoTaq® PCR Core System II showing a product of 323 bp; lane2: negative control with no template DNA; lane 3: primer OPA-05; lane 4: primer OPA-08; lane 5: primer OPA-09; lane 6: primer OPC-05; lane 7: primer OPC-09.

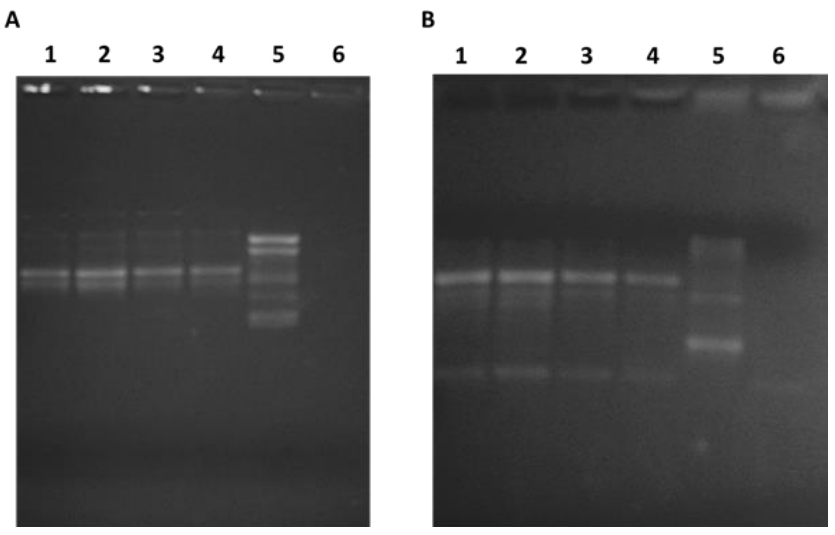

Fig. 2. RAPD analysis of "Maggie" samples. DNA from each sample was isolated and amplified as described in Materials and Methods. Part **A** shows DNA samples amplified with primer OPA-05, and Part **B** shows DNA samples amplified with primer OPA-08. Lane 1: "Maggie" grown at Florida Southern College; lane 2: "Pacific;" lane 3: "Maggie" from Peggy Martin; lane 4: "Kakinada Red;" lane 5: *Rosa laevagata*; lane 6: negative control (no template DNA added).

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