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RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS OF THE MUSK ROSES (*ROSA MOSCHATA*)

CARMELLA FREDERICK, AMANDA WAGNER AND NANCY MORVILLO[1]
Florida Southern College
111 Lake Hollingsworth Dr.
Lakeland, FL 33801

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Abstract. **The genetics of different musk rose (*Rosa moshcata* Herrmann) varieties were compared via randomly amplified polymorphic DNA (RAPD) analysis. All varieties were found to be extremely similar, if not identical, except for "Bremo". Bremo's banding patterns for all primers deviate enough from the other observed patterns to suggest it should not be classified as a musk variety. Despite morphological differences, the data suggest that small genetic mutations, rather than major genomic alterations, account for variations among the other musk varieties. The results also give considerable evidence that the varieties have been propagated vegetatively, probably from a single original clone.**

The true musk roses (*Rosa moschata* Herrmann) are historically important in that they, along with one other rose variety ('Autumn Damask'), are the only European roses that show repeat flowering. Today, all rose cultivars are bred to include this trait. As the parentage of 'Autumn Damask' includes the musk roses (Thomas, 1994; Iwata et al., 2000), it is likely that the gene(s) responsible for repeat flowering in all roses of European decent are originally from the musk roses.

The musk roses are native to North Africa, and the area from southern Europe to western Asia. They were brought to England during the early Elizabethan age (Thomas, 1994). However, the musk rose has often been a victim of mistaken identity since this time. The musk rose referred to by Shakespeare was probably *R. arvenis* (Thomas, 1994). Also in the United States, *R. moschata nepalensis* Lindley (aka. *R. brunonii*) was falsely identified as the true musk rose (Manners, 2000). *R. brunonii* is a more vigorous rose and easier to propagate, and therefore nurseries grew it as a true musk (Thomas, 1994).

The Burwell Family and their close friends most likely propagated the true *R. moschata* in the United States (Butler, 2002). All of the currently identified musk roses have been rediscovered since 1970, beginning with the "Burwell School" at Hillsborough School for Girls. Two more specimens, "Elmwood Single" and "Elmwood Double," were found in the Burwell plot of the Elmwood Cemetery in Charlotte, North Carolina. The "Crenshaw" musk was found in the Crenshaw plot of the Hollywood Cemetery in Virginia. "Bremo" was found in 1988 at Bremo, forty miles from Monticello, in Virginia. In 1985, "Saluda" was discovered in Saluda, South Carolina. The other varieties of musk roses ("Graham Thomas's," "Gate Tayloe" and "Temple") were rediscovered in England (Butler, 2002). All of these varieties, with the exception of the Burwell School and Bremo musk, have been growing on the campus of Florida Southern College, Lakeland, Florida, for at least seven, and in some cases, nearly twenty years (Manners, 2000).

R. moschata has three main phenotypes: single (*R. moschata moschata*), double (*R. moshcata plena*), and very double (Manners, 2000). These phenotypes are similar in color, leaf structure, and size of rosebush, while they differ in flower appearance. The single musk has five petals and many reproductive organs. The double and very double musks have many petals and almost a complete absence of reproductive organs. The double musk has central petalloids that shrivel and turn brown in the sun, while the very double does not exhibit this "frying" phenomenon. Although three different forms are apparent, the exact history of these roses is not known. There is no historical record of whether these phenotypes are different subspecies, hybrids, or three different cultivars resulting from vegetative propagation.

Due to the absence of precise historical records, it was decided to examine the musk roses on a genetic level for their

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[1]Corresponding author.

relationships to each other (Faria et al., 2000; Walker and Werner, 1997). A specific type of the polymerase chain reaction (PCR), known as randomly amplified polymorphic DNA (RAPD) analysis, was done on the musk roses to analyze the genomic DNA from each variety. The results indicate the genomic DNA is similar, if not identical, in all varieties except one, and therefore lend support to a single musk plant that has been propagated vegetatively and has acquired small mutations to produce the observed phenotypic variations.

Materials and Methods

Plant Material. Small, unopened leaves were collected from varieties of musk rose (*R. moschata* Herrmann) and from an unrelated rose species (*R. laevigata* Michaux) used as a control. All varieties are grown on the Florida Southern College Campus, with the exception of "Burwell School" (samples obtained from R. Knopf, Charleston, South Carolina). Samples were used either fresh or stored at -25 °C.

DNA Isolation. Genomic DNA was isolated as described by Walker and Werner, (1997) with modifications. Three leaves were quick frozen in dry ice/ethanol bath and then ground in a chilled mortar and pestle. Ground tissue was mixed with 750 µL of lysis buffer (100 mM sodium acetate, 50 mM Na_4EDTA, 500 mM NaCl, 2% PVP40, 1.4% SDS, pH 5.5, with 60 mM β-mercaptoethanol added immediately prior to use). Samples were incubated at 65 °C for 15 min and centrifuged for 10 min at 14,000 g_n. One-third volume of 3M potassium acetate was added to the supernatant and incubated on ice for 15 min. The sample was centrifuged for 10 min at 14,000 g_n. DNA was precipitated by the addition of 0.6 volume cold isopropanol to the supernatant. The sample was incubated at 4 °C for 30 min and then centrifuged for 20 min at 14,000 g_n. The pellet was washed in 70% ethanol and resuspended in 100 µL of TE/RNase buffer (10 mM Tris, 1 mM Na_4EDTA, pH 8.0, and 10 µg/mL $RNase^{-1}$). Samples were run on a 0.7% agarose gel and concentrations were estimated by comparison with known DNA standards.

PCR Conditions. RAPD analysis was performed using procedures described by Walker and Werner (1997). PCR reactions contained 1.0 × Taq buffer (Roche Molecular Biochemicals, Indianapolis, IN), 2.5 mM $MgCl_2$, 200 µM each of dATP, dGTP, dCTP, dTTP, 0.2 µM of each primer, 1 unit Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN), and 0.5 ng of genomic DNA, in a final volume of 50 µL. Primers (OPA-05, OPA-08, OPA-09, OPC-05, and OPC-09) were obtained from Operon Technologies, Inc., Alameda, CA. The sample was overlaid with 50 µL of mineral oil. A model PTC150 thermal cycler (MJ Research Inc., Watertown, MA) was programmed 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, held at 4 °C. A 20 µL portion of the PCR samples was run on a 1.5% agarose gel, stained with 0.5 $\mu g \cdot mL^{-1}$ ethidium bromide, and viewed on an ultraviolet transilluminator. Gels were photographed and the resulting banding patterns were compared. Analysis was performed at least twice with each variety of musk rose and each primer.

Results and Discussion

PCR has become an invaluable tool for amplifying specific regions of DNA for analysis. With the RAPD procedure, however, the exact sequences being amplified are not known. This allows for comparison of many random sequences between species and detection of polymorphisms among different individuals within a species. If the resulting patterns from the RAPD analysis are identical or nearly so, this is an indication that there are no gross genomic differences among samples. There may be smaller variations, such as point mutations or deletions of small regions. Therefore, RAPD analysis is a useful tool in determining whether genomes are similar enough to be considered as belonging to one species, or if there is enough variation to conclude the samples belong to different species (Welsh and McClelland, 1990).

The three different cultivars of musk roses, represented by nine different varieties (Table 1), were analyzed using five different sets of primers (OPA-05, OPA-08, OPA-09, OPC-05, and OPC-09). An example of the RAPD results is shown in Fig. 1. The banding pattern from each musk is nearly, if not entirely, identical to all the other musk varieties, and is completely different from that of the *R. laevigata* control (lane 2). The only exception is the "Bremo" musk sample (lane 11). Similar to the *R. laevigata* control, the Bremo pattern is unique and indicates this sample is unrelated to the other musk samples.

The results were similar with all five primers used. The only major exceptions occurred with the OPA-08 primer (Fig. 1B; Table 2). In the analysis with this primer, several polymorphic bands were detected, but none could be correlated with a particular phenotype. Although some bands varied among samples, the remaining bands were consistent among all samples, again with the exception of Bremo.

The similar profiles of the true musk roses indicate that the phenotypic differences mentioned earlier are the result of point mutations or small genomic alterations and do not indicate that musk roses belong to different species. The phenotypic differences are probably not accounted for by the polymorphisms noted using the OPA-08 primer, however, as the detected polymorphisms appear to have no correlation to the phenotypes discussed. It should also be noted at this point that all double varieties frequently sport to single flowers, singles rarely sport to doubles, and doubles rarely sport to the very double phenotype (Manners, 2000), again indicating the different flower phenotypes are due to "small" mutations. Therefore, the traditional taxonomy is not valid, and the different musk cultivars do not deserve subspecies rank.

The Bremo musk was the only one of the musk roses to exhibit a completely different pattern. The genetic profile of the Bremo musk led us to conclude that Bremo is not a true musk. The RAPD results support earlier questions raised

Table 1. *Rosa moschata* varieties used in this study, with specific phenotypes indicated. All samples, with the exception of "Burwell School" and "Bremo," have been grown on the campus of Florida Southern College, Lakeland, Florida, for at least seven years.

Variety	Phenotype
Burwell School	double
Bremo	double
Crenshaw	double
Elmwood Double	double
Elmwood Single	single
Gate Tayloe	double
Graham Thomas's	single
Saluda	double
Temple	very double

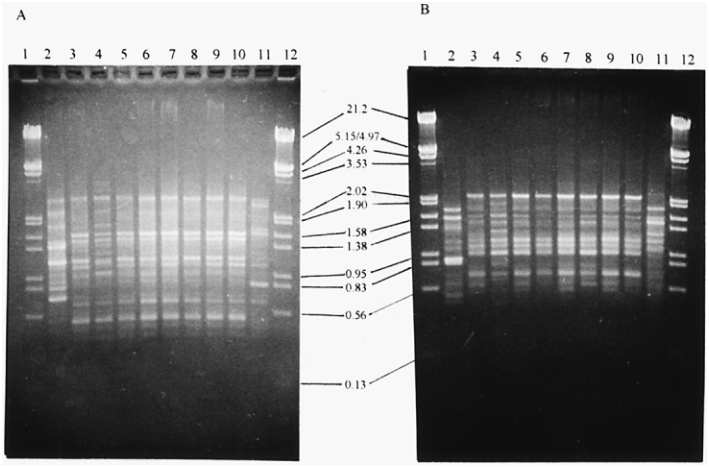

Fig. 1. Banding patterns of the musk roses resulting from RAPD analysis. The DNA from the nine varieties of *R. moschata* and the control sample, *R. laevigata*, was isolated and subjected to RAPD analysis as described in Materials and Methods. Shown are photographs of the resulting gels. Primers used were: A = OPC-05 and B = OPA-08. Samples are as follows: Lanes 1 and 12: Lambda marker DNA cut with *HindIII* and *EcoRI*; lane 2: *R. laevigata*; lane 3: Graham Thomas's; lane 4: Burwell School; lane 5: Elmwood Single; lane 6: Elmwood Double; lane 7: Gate Tayloe; lane 8: Saluda; lane 9: Crenshaw; lane 10: Temple; lane 11: Bremo. Numbers to the sides of gels indicate size of marker fragments in kilobase (kb) pairs.

about Bremo's classification. Its physical appearance and flowering and growth characteristics are unlike those of the other musk varieties (M. Manners, Florida Southern College, pers. comm.). The Bremo sample grown on the grounds of Florida Southern College was obtained recently from The Center for Historic Plants at Thomas Jefferson's Monticello estate and may not be the true Bremo musk rose. Further investigations are proceeding to answer this question.

In conclusion, the RAPD profiles of all the musk roses, with the exception of "Bremo," appear identical to each other and indicate the genomes of all these varieties are extremely similar. This would place them genetically into the same species. The phenotypic variations seen are probably the result of small mutations, undetectable by the RAPD procedure. Research is continuing into the actual causes of some of these differences.

Table 2. Polymorphic bands appearing with OPA-08 primer. The symbol "+" indicates the band in question was present, whereas "-" indicates it was not present. The Bremo sample is not included in this data.

	Band size (kb)					
Variety	1.90	1.30	0.08	0.65	0.60	0.50
Burwell School	+	+	+	+	-	-
Crenshaw	-	-	-	-	+	+
Elmwood Double	-	-	-	-	-	-
Elmwood Single	-	-	-	-	+	+
Gate Tayloe	-	-	-	-	+	+
Graham Thomas's	-	-	-	-	+	-
Saluda	-	-	-	-	+	-
Temple	-	-	-	-	+	+

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