Development and characterization of microsatellite loci for the endangered scrub lupine, *Lupinus aridorum* (Fabaceae)¹

ANGELA RICONO², GLEN BUPP³, CHERYL PETERSON³, SCHYLER O. NUNZIATA⁴, STACEY L. LANCE⁴, and CHRISTIN L. PRUETT²,⁵

¹Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901 USA; ²Rare Plant Conservation Program, Bok Tower Gardens, Lake Wales, Florida 33853 USA; and ³Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina 29802 USA

- **Premise of the study:** Microsatellite primers were developed in scrub lupine (*Lupinus aridorum*, Fabaceae), an endemic species to Florida that is listed as endangered in the United States, to assess connectivity among populations, identify hybrids, and examine genetic diversity.
- **Methods and Results:** We isolated and characterized 12 microsatellite loci polymorphic in scrub lupine or in closely related species (i.e., sky-blue lupine [*L. diffusus*] and Gulf Coast lupine [*L. westianus*]). Loci showed low to moderate polymorphism, ranging from two to 14 alleles per locus and 0.01 to 0.86 observed heterozygosity.
- **Conclusions:** These loci are the first developed for Florida species of lupine and will be used to determine differentiation among species and to aid in conservation of the endangered scrub lupine.

**Key words:** Fabaceae; Florida; Lupinus; microsatellite; PCR primers.

Scrub lupine (*Lupinus aridorum* McFarlin ex Beckner) is an endangered plant restricted to the Winter Haven and Mount Dora ridge systems in central Florida that has declined primarily due to habitat loss (USFWS, 1987). Polymorphic genetic markers are needed to answer questions about genetic diversity and connectivity among *L. aridorum* populations, genetic relatedness among Florida lupine species including the closely related Gulf Coast lupine (*L. westianus Small*), and hybridization between sympatric populations of *L. aridorum* and sky-blue lupine (*L. diffusus Nutt.*; Bupp, 2013).

**METHODS AND RESULTS**

Total genomic DNA was extracted from leaf samples of two individuals from *L. aridorum* populations using a QIAamp DNA Mini Kit (QIAGEN, Valencia, California, USA). Using a Covaris S220 (Woburn, Massachusetts, USA), we prepared an Illumina paired-end shotgun library by shearing 1 μg of DNA as described in the Illumina TruSeq DNA Library Kit (Illumina, San Diego, California, USA) and using a multiplex identifier adapter index. Illumina sequencing, with 100-bp paired-end reads, was conducted on a HiSeq 2000 (illumina) to examine five million reads and extracted the reads that contained di-, tri-, tetra-, penta-, and hexanucleotide microsatellites. Positive reads were batched to the program Primer3 (version 2.0.0; Rozen and Skaletsky, 1999) for primer design. We selected loci for primer sequences that only occurred one time in the five million reads to avoid problems with copy number of the sequence in the genome. Ninety loci of the 1740 loci that met this criterion were chosen. One primer from each pair was modified on the 5’ end with an engineered sequence (CAG tag 5’-CAGTCGGGCGTCATCA-3’) to enable use of a third primer in the PCR that was fluorescently labeled with one of three dyes (6-FAM, NED, or VIC; Applied Biosystems, Culver City, California, USA). Primer pairs were tested for amplification and polymorphism using DNA obtained from four individuals. Amplifications were in 20-μL volumes (250 μg/mL bovine serum albumin [BSA], 2 μL 10x Buffer B, 25 mM MgCl₂, 5 μM unlabeled primer, 0.5 μM tag-labeled primer, 5 μM universal dye-labeled primer, 2.5 mM dNTPs, 0.5 units Taq DNA polymerase [Fisher Scientific, Pittsburgh, Pennsylvania, USA], and 20 ng DNA template) using a Bio-Rad MyCycler (Hercules, California, USA) thermal cycler. We used touchdown cycling conditions to amplify DNA and to attach the universal dye-labeled primer. Parameters consisted of an initial denaturation step of 2 min 30 s at 95°C; followed by 20 cycles of 95°C for 20 s, 65–50°C annealing temperature for 20 s (decreasing 0.5°C per cycle), and extension step of 72°C for 30 s; followed by 15 cycles of 95°C for 20 s, 55–45°C for 20 s, and 72°C for 30 s. Cycles were followed with a final extension step of 72°C for 10 min. Amplifications were run on an ABI3730XL sequencer (Applied Biosystems).

Twelve of the tested primer pairs amplified high-quality PCR product that exhibited polymorphism in *L. aridorum*, *L. diffusus*, or *L. westianus* (Table 1). We then assessed the variability at these loci using 19–22 individuals of *L. aridorum*, 9–22 *L. diffusus*, and 12–20 *L. westianus* (Table 2). Alleles were scored using GeneMapper software (Applied Biosystems). We evaluated the number of alleles per locus, observed heterozygosity, and expected heterozygosity and tested for Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using Arlequin version 3.5 (Excoffier et al., 2005). Sequences of raw paired-end reads are available in the SRA database of the National Center for Biotechnology Information (bioproject no. PRJNA274660) and as Appendix S1. Vouchers of leaves collected for this study were deposited at Bok Tower Gardens, Lake Wales, Florida (Table 2).

We found that the number of alleles per locus ranged from one to seven for *L. aridorum*, one to nine for *L. westianus*, and one to 14 for *L. diffusus*. For...