THE SAME BUT DIFFERENT: MONOMORPHIC MICROSATELLITE MARKERS AS A NEW TOOL FOR GENETIC ANALYSIS

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Methods and Results: From a set of microsatellite markers, a monomorphic microsatellite locus developed for the palm species Butia eriospatha was used to elucidate whether there are polymorphic sites in its flanking regions. DNA sequences long were obtained. Aligned sequences show variation at 17 polymorphic sites with both insertions and nucleotide substitutions. Fourteen distinct sequences (alleles) among 22 individuals were identified. The percent sequence difference varied from 0.0 to 5%, indicating that there is significant variation among sequences.

Conclusions: Due to significant levels of information and sequence diversity on a simple sequence repeat (SSR) locus of identical size, our study highlights that this molecular marker class can be a useful tool for population genetics and evolutionary studies for many plant species.

Key words: Butia eriospatha; monomorphic SSR; sequence variation.

Many different methods can be employed to reveal molecular markers. PCR-based assays of simple sequence repeat loci (SSRs or microsatellites) have become the most popular and powerful of the current methods for identifying highly polymorphic genetic markers. Polymorphisms in SSRs, which are abundant in the genomes of many taxa, are thought to result from salutary replication, unequal crossovers, or possibly gene conversion (Lewin, 1997) and often exhibit high levels of heritable variation in the number of repetitions of a particular motif. Traditional genetic surveys of microsatellite loci capitalize on SSR variation because it is easy to score DNA fragments by size. Length variation is usually the sole and most conspicuous criterion employed to characterize allelic diversity at loci displaying variable numbers of tandem repeats. Unfortunately, SSR loci that are monomorphic in length have been excluded from both evolutionary and population genetics approaches due to their apparent lack of genetic variability. Furthermore, since microsatellite loci were discovered (Litt and Luty, 1989; Tautz, 1989), only recently have periodicals (e.g., American Journal of Botany) begun to accept publications that include monomorphic loci.

Beyond variation by size at SSR loci, previous reports of DNA sequence analysis have noted that nucleotide sequence variation in flanking regions of polymorphic loci have hinted at the mutational complexity and evolutionary diversity that can underlie conventionally detected size differences among microsatellite alleles (Estoup et al., 1995; Ortí et al., 1997). From this point of view, published monomorphic loci can be used in addition to length variation. Therefore, the goals of this study are to address some of the questions that have arisen regarding the use of monomorphic loci: How much variation remains hidden at a monomorphic SSR locus? Is this molecular marker class suitable for genetic analysis? The voucher palm Butia eriospatha (Mart. ex Drude) Becc. was considered as a model species to elucidate the answers to these questions because palms appear to have lower rates of molecular change (Smith and Donoghue, 2008).

METHODS AND RESULTS

From a set of microsatellite markers previously isolated for the palm species B. eriospatha (Nazareno et al., 2011), a dinucleotide (GT), monomorphic microsatellite locus (BUT10) was used to elucidate if there are polymorphic sites in its flanking regions. To ratify the status of the monomorphic locus, the current study initially characterized 100 individuals from four populations for the BUT10 locus. The PCR and profile used to amplify the BUT10 locus are described at Nazareno et al. (2011). PCR products were denatured and separated with 10% denaturing polyacrylamide (39:1 acrylamide to bisacrylamide) gels stained with silver nitrate. Gels were run with 1× TBE buffer (90 mM Tris, 92 mM boric acid, and 2.5 mM EDTA) on a vertical electrophoresis at a constant electric current (21 mA for each gel) for six hours. Allele sizes were estimated by comparison with a 10 bp DNA ladder standard (Invitrogen, Carlsbad, California, USA). All samples contained fragments with the same length (~132 bp).

To detect sequence variation, we selected all 29 B. eriospatha plants from one natural population out of the original sample. Furthermore, one adult plant from a population (designated as Fy 200 km away, located in Santa Catarina State, southern Brazil, was added to the analysis. For sequence procedures, the PCR products of the BUT10 locus were stained with Gel-Red, and the presence of a single clear band was verified in 1.5% agarose gel. The gel was run with 1× TBE buffer on a horizontal electrophoresis at a constant voltage (120 V) for one hour, and was viewed under ultraviolet light. The bands were extracted from the gel, purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin, USA), and ligated into pGEM-T Easy Vector System I (Promega). The ligation reaction consisted of a final volume of 20 µL, containing 3 U T4 DNA Ligase (Promega), 10 mM ligase buffer (300 mM Tris–HCl pH 7.8, 100 mM MgCl2, 100 mM DTT, and 10 mM ATP), 50 ng of plasmids, and 20 ng...
Due to the observed sequence variation within a population of *B. eriospatha*, a taxon with an apparently low evolutionary rate, this study demonstrates that monomorphic molecular markers can be suitable for genetic population and phylogenetic analyses in many plant species. We plan to use this molecular marker to evaluate the genetic divergence of *B. eriospatha* among populations in the Atlantic Rainforest, the native region of this vulnerable palm species.

**LITERATURE CITED**


APPENDIX 1. Specimens used in this study. Specimens were deposited at the herbarium of the Universidade Regional de Blumenau (FURB). Information presented: taxon, voucher specimen, collection locale, GenBank accession numbers.