Primer Note

Isolation of 91 Polymorphic Microsatellite Loci in the Western Mediterranean Endemic Carex helodes (Cyperaceae)

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• Premise of the study: Microsatellite primers were developed for Carex helodes (Cyperaceae), a western Mediterranean endemic that is locally distributed in southern Portugal and southwestern Spain and rare in northern Morocco.

• Methods and Results: One hundred nine nuclear microsatellite markers were developed using a shotgun pyrosequencing method, resulting in 91 polymorphic and 18 monomorphic loci when tested using 19 individuals sampled from five populations from Portugal, Spain, and Morocco. Loci averaged 3.23 alleles per locus (SD = 1.15). In a single population (Cortelha population, Portugal), the 34 most polymorphic loci showed a mean observed heterozygosity of 0.357 (SD = 0.292) and mean expected heterozygosity of 0.384 (SD = 0.255).

• Conclusions: Next-generation sequencing allowed us to develop a high number of genetic markers with levels of polymorphism adequate to study gene flow among populations. However, when genotyping the individuals within a population, we found low levels of variation.

Key words: Carex helodes; Cyperaceae; endemism; sedge; shotgun sequencing; simple sequence repeat (SSR) marker.

Carex helodes Link (sect. Spirostachyae (Drejer) L. H. Bailey, Cyperaceae) is a diploid, wind-pollinated, perennial herb with a minimum generation time of two years. The species is endemic to the western Mediterranean, being locally distributed in southern Portugal and southwestern Spain, and rare in northern Morocco (Escudero et al., 2008a). This sedge occurs in temporarily inundated acidic soils in open cork oak woodlands. Despite its well-characterized morphology, C. helodes has been misidentified as C. laevigata Sm. by some authors (see Luceño et al., 2009). Recent cytotaxonomic and nuclear-and plastid-based phylogenetic studies have revealed the monophyly of C. helodes populations and its taxonomic independence within sect. Spirostachyae (Escudero et al., 2008a, 2008b; Escudero and Luceño, 2009). Carex helodes is an endangered species in Spain because the extent of its severely fragmented occurrence is less than 100 km², with continued loss of area and habitat quality (Moreno, 2008; Bañares et al., 2010).

Our aim is to develop molecular markers for further studies of gene flow among and within populations. Nuclear microsatellites have been proven to be highly variable and very suitable to the study of recent gene flow between populations (Ouborg et al., 1999). To accomplish our task, we isolated and characterized 109 nuclear microsatellites.

Methods and Results

We extracted genomic DNA using a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). We used ~5 μg from one C. helodes individual collected in Madroñalejo (Aznalcóllar, Seville, Spain; see Appendix 1 for GPS coordinates and voucher specimens) to construct a shotgun genomic library that was sequenced on 1/4th of a plate using 454 GS FLX Titanium chemistry (Roche Applied Science, Indianapolis, Indiana, USA) at the University of Arizona Genetic Core (Tucson, Arizona, USA). We generated 108.3 Mb of quality-filtered data, distributed over 221,198 unique reads with an average length of 490 bp after quality filtering (quality score [Q] ≥ 20 using a 10-bp sliding window). We searched for all possible microsatellite loci containing at least six perfect repeats for hexa-, penta-, tetra-, and trinucleotides or 12 perfect repeats for dinucleotides and designed primers using the software QDD version 3 (Meglécz et al., 2014). We used the unique reads as input to detect microsatellite sequences. The reads were used to build contigs using QDD version 3 (default options were used; sequence set limit of 80 bp, 95% minimum identity between two sequences to make a consensus, and 66% as the proportion of sequences that must have the same base on the aligned sites to accept it as a consensus). For primer design, the default options were also used but the minimum size of the PCR product was set to 100 bp and the maximum to 450 bp.

We found a total of 3985 microsatellite loci, including 51 hexa-, 58 penta-, 78 tetra-, 406 tri-, and 3392 dinucleotide loci. We selected 27 hexa-, 26 penta-, 23 tetra-, 206 tri-, and 152 dinucleotide loci that met our criteria (at least 12 repeats for dinucleotides and six for the rest) and tested a total of 132 loci. Specifically, we tested the eight hexa-, eight penta-, eight tetra-, 18 tri-, and 90 dinucleotide loci with the highest numbers of repeats (Table 1).

For primer testing, DNA was isolated from silica gel-dried leaves using a modified cetyltrimethylammonium bromide (CTAB) extraction method.