PRIMER NOTE

New microsatellite markers for the endemic Mediterranean seagrass Posidonia oceanica

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Abstract

The seagrass Posidonia oceanica is endemic to the Mediterranean Sea, where it plays an important role in coastal ecosystem dynamics. Because of this important role and concerns about the observed regression of some meadows, population genetic studies of this species have been promoted. However, the markers used until now were not polymorphic enough to efficiently assess the level and spatial pattern of genetic variability. Hypervariable molecular markers were obtained by screening a genomic library enriched for microsatellite dinucleotide repeats. Among 25 primer pairs defined, eight amplified polymorphic microsatellites with an encouraging level of variability at the two geographical scales sampled.

Keywords: clonal plant, dinucleotides, microsatellites, Posidoniaceae, seagrass

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The endemic seagrass Posidonia oceanica plays an important role in coastal ecosystem dynamics in the Mediterranean Sea. However, anthropogenic perturbations such as pollution, modifications of coastal areas or introduction of new species have led to widespread regression of the species (e.g. Duarte 2002). The threats to this species have led to an interest in genetic studies, aimed at elucidating the genetic variability and the level of gene flow within and between populations, as a basis for conserving and managing P. oceanica along Mediterranean coasts.

Posidonia oceanica is a clonal plant that can reproduce either sexually by seed production or asexually by vegetative propagation of rhizomes. The first studies based on allozymes and RAPD techniques revealed very low polymorphism, preventing the efficient study of genetic variability and gene flow (Capionont et al. 1996; Procaccini & Mazella 1996). Six polymorphic microsatellites were isolated (Procaccini & Waycott 1998), mostly presenting trinucleotide (but sometimes irregular) motifs, which were limited in their capacity to resolve genetic structure relative to that expected from hypervariable markers (Procaccini & Mazella 1998; Procaccini et al. 2001). In order to determine whether the low level of genetic variability of the species suggested by the use of these markers is unambiguously attributable to a biological feature of this clonal species, or, rather, reflects the limitations of the microsatellites isolated, we isolated new microsatellites with dinucleotide repeats.

Genomic DNA was isolated from a fresh meristematic portion of P. oceanica leaves, following standard cetyltrimethyl ammonium bromide (CTAB) extraction procedures, and was digested with Af1 (Rsal) (Amersham Pharmacia Biotech). Total digested DNA was purified and ligated into annealed Af1 adaptors (AdapF: 5'-TCTTGCTTACGCGTGGACTA-3' and AdapR: 5'-TAGTCCACGCGTAAGCACA-3'). The enrichment procedure used streptavidin-coated magnetic particles (MagneSphere, Promega) and followed the protocol from Billote et al. (1999) based on modifications from the original (Kijas et al. 1994). A 5'-biotinylated with a probe 3'-dideoxyC was used to avoid production of chimeric clones (Koblízková et al. 1998). The enriched single-stranded DNA was amplified by polymerase chain reaction (PCR) using the AdaptF as a primer to recover double-stranded DNA. The double-stranded DNA was quantified and ligated into the pGEM-T Easy vector (Promega). The ligation was transformed into Escherichia coli cells (strain DH5α). About 730 colonies containing an insert of P. oceanica were transferred from the agar plates.
Table 1 Characterization of eight microsatellite loci, including locus name, repeated microsatellite motif, GenBank accession number, annealing temperature (T_a), approximate allele size (bp) and number of alleles detected on a sample of nine populations, number of alleles and expected (H_E) and observed (H_O) heterozygosity estimated in one natural population are given for each locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Microsatellite motif</th>
<th>Primer sequences (5’-3’)</th>
<th>GenBank accession no.</th>
<th>T_a (°C)</th>
<th>MgCl₂ (µM)</th>
<th>Approx. size range of alleles (bp)</th>
<th>No. of alleles in 9 meadows of <em>P. oceanica</em></th>
<th>No. of alleles in Formentera meadows</th>
<th>H_E</th>
<th>H_O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Po-5</td>
<td>(GA)₂₀</td>
<td>CACAATGCCCCCGTTAGCA</td>
<td>AF547650</td>
<td>64-52</td>
<td>2.5</td>
<td>164-188</td>
<td>6</td>
<td>4</td>
<td>0.43</td>
<td>0.48</td>
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<tr>
<td>Po5-10</td>
<td>(GA)₁₃</td>
<td>GTGGTTGCGTGGCTCGTTTG</td>
<td>AF547651</td>
<td>55</td>
<td>1.5</td>
<td>151-179</td>
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<td>4</td>
<td>0.60</td>
<td>0.94</td>
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<tr>
<td>Po5-39</td>
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<td>ATGAGACTCCCCACAATTACA</td>
<td>AF547652</td>
<td>54</td>
<td>1.5</td>
<td>170-182</td>
<td>6</td>
<td>5</td>
<td>0.57</td>
<td>0.37</td>
</tr>
<tr>
<td>Po5-40</td>
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<td>CATGGGATGCTGCTCCCTTCG</td>
<td>AF547653</td>
<td>55</td>
<td>1.5</td>
<td>186-258</td>
<td>21</td>
<td>8</td>
<td>0.75</td>
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<tr>
<td>Po5-49</td>
<td>(GA)₁₆</td>
<td>ATGCAAAAGGAAGACACTCA</td>
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<td>55</td>
<td>1.5</td>
<td>218-242</td>
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<td>2</td>
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<td>0.11</td>
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<td>(GA)₂₀</td>
<td>GGGATTCCCTCCTCGCC</td>
<td>AF547655</td>
<td>64</td>
<td>1.5</td>
<td>137-171</td>
<td>13</td>
<td>3</td>
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<td>0.77</td>
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<tr>
<td>Po4-3</td>
<td>(GA)₁₀</td>
<td>AAGCCGCGCTTAACTAAA</td>
<td>AF547656</td>
<td>51</td>
<td>1.5</td>
<td>158-178</td>
<td>7</td>
<td>7</td>
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<tr>
<td>Po4-36</td>
<td>(AG)₂₀(AG)₂₂</td>
<td>AAGCCGCGCTTAACTAAA</td>
<td>AF547657</td>
<td>53-50</td>
<td>1.5</td>
<td>185-197</td>
<td>4</td>
<td>3</td>
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</tbody>
</table>
into microplates containing 150 μL LB-Ampicillin solution, and were incubated for 4 h at 37 °C. Subsequently, the grown colonies were diluted 5x in Sigma Water on new microplates and heated for 10 min to induce cell lysis. The size of inserts was estimated by agarose gel electrophoresis of PCR products of standard M13 and reverse primers flanking pGEM-T easy polylinker. Gels were photographed and Alkaline Southern transferred onto nylon membranes (Hybond-N; Amersham Pharmacia Biotech). Hybridization was carried out with a 32P-radiolabelled (CT)15 probe (Cregan et al. 1994). About 20% of the clones sequenced contained microsatellites. Plasmids from the positive clones were extracted using the Wizard Plus miniprepDNA (Promega) purification system. From these, 73 were sequenced commercially (MWG-Biotech AG) and 25 primer pairs were designed using primer3 software (Rozen & Skaletsky 2000).

Microsatellite polymorphism was analysed by specific PCR at two population levels. Forty individuals randomly collected inside an apparently homogeneous 800 m² area of a meadow located at 10 m depth in the island of Formentera (Balearic Islands, Spain) were first analysed to assess the level of intrapopulation variability (number of alleles, heterozygosity and clonal resolution), and then five natural populations, except specific primer pairs were designed using (described above), and the same PCR conditions as those of the 25 loci isolated, eight were polymorphic, exhibiting from two to 12 alleles (Table 1). The number of alleles and the level of heterozygosity were relatively high for most of the markers (Table 1) and allowed detection of 24 genets among the 35 samples analysed from the natural population of Formentera. No deviation from Hardy–Weinberg equilibrium and no linkage disequilibrium were detected when excluding from the sample the replicates of the same clone (i.e. ramets issued of the same genet).

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References