Isolation and Characterization of Nine Microsatellite Markers for Cymbidium sinense

Kuaifei Xia, Xiulin Ye, and Mingyong Zhang
South China Botanical Garden, the Chinese Academy of Sciences, Xingkelu 723, Guangzhou 510650, China

Abstract. Microsatellite-enhanced genomic library of Cymbidium sinense was constructed using repeat-enrichment method with biotin-labeled oligos and streptavidin magnetic beads. Twenty-five microsatellite loci were isolated from a microsatellite-enhanced genomic library of Cymbidium sinense. Nine of these loci displayed genetic diversity that ranged from one to 15 alleles per locus, and the observed heterozygosity of six polymorphic loci ranged from 0.18 to 0.90 with an average of 0.47 (0.29 sd) in a sample of 30 individuals. Eight of these loci were successfully amplified in at least one of the following species: Paphiopedilum hissatusimum, P. wardii, P. armeniacum, or P. micranthum. The study provides a base for molecular breeding and assessment of germplasm resources of the Cymbidium sinense and the results suggest that the microsatellite loci developed from C. sinense may have a broad applicability within the Orchidaceae family.

Microsatellites, also known as single sequence repeats (SSRs), are a small array of one to six tandemly arranged bases spread throughout the genomes (Dietrick et al., 1992). The development of SSR-based markers has become increasingly accessible in recent years mainly as a result of novel library enrichment strategies and rapid fluorescence-based automatic sequencing technologies (Powell et al., 1996). SSRs have now been recommended as an effective genetics marker to be used in the construction of genetic linkage maps, molecular tagging, molecular marker-assisted selection, parentage test, fingerprint identification, population genetics, and the resources conservation and management (Dick and Hamilton, 1999; Dietrick et al., 1992; Jarne and Lagoda, 1996).

The genus Cymbidium is one of the favorite orchids in the Chinese culture (Hu, 1977). Cymbidium sinense, a terrestrial orchid whose native habitat spreads from India through Thailand and into South China, has been cultivated for several centuries in China and Japan (Du Puy and Cribb, 1988). The resources of this species decreased dramatically recently and it becomes one of the rare and endangered plants in the world. Despite their significance, many aspects of rare and endangered plants in the world.

Recombinant plasmids were identified by ORT cloning according to the manufacturer's protocol. Synthes.

Received for publication 14 May 2008. Accepted for publication 10 June 2008.
This research was supported by Technology of Guangdong province (2006A20101007, 2006A20201001), the Knowledge Innovation Program of the Chinese Academy of Sciences (200715), and SRF for ROCS, SEM.
* To whom reprint requests should be addressed; e-mail zhangmy@scbg.ac.cn

Additional index words. Cymbidium sinense, microsatellite, molecular marker, Orchidaceae
Bonferroni procedure (Rice, 1989). Five loci (Cym 9, Cym 15, Cym 18, Cym 344, and Cym 371) showed significant deviations from the HWE (adjusted $P = 0.0083$; Table 1) in the screened samples, and the tested population was not within HWE when combined over the six loci ($F_{	ext{is}} = 0.18, P < 0.001$). The observed heterozygote deficiencies and deviations from HWE in this study may be the result of sample mixture (Wahlund effect) or the presence of null alleles and genetic drift and/or inbreeding in an isolated ex situ population (Caughley, 1994; Mitton and Grant, 1984). Highly significant ($P < 0.001$) linkage disequilibrium was exhibited in the sampled population between locus Cym 9 and Cym 15 in the 15 tests for linkage disequilibrium performed for all possible pairwise comparisons of the sampled loci.

The 11 loci were also screened in 30 individual genomic DNAs of an ex situ conservation population of *Paphiopedilum hissutissimum* in South China Botanical Garden using the PCR conditions optimized for *C. sinense*. The results of cross-amplification are summarized in Table 1. Four of these loci were successfully amplified in the species tested, and one locus (Cym 18) showed polymorphism within the tested population with an observed heterozygosity of 0.12 and an expected heterozygosity of 0.18. Additionally, all these loci were also screened in two individuals of the species *P. wardii*, *P. armeniacum*, and *P. micranthum*, respectively, using the optimal PCR conditions for *C. sinense*. Seven of these loci were successful.

### Table 1. Summary of the developed microsatellite markers from *Cymbidium sinense*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank accession no.</th>
<th>Repeat motif</th>
<th>Primer sequences (5’ → 3’)</th>
<th>Species</th>
<th>R (bp)</th>
<th>$A_{O}$/$A_{E}$</th>
<th>$T_{d}(°C)$</th>
<th>$P$</th>
<th>Fis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cym 9</td>
<td>DQ501379 (GA)$_{33}$</td>
<td>F: GCCTACGTGAATATGATAAA</td>
<td>C.s.</td>
<td>105–172</td>
<td>12</td>
<td>0.93/0.71</td>
<td>53</td>
<td>0.0000</td>
<td>0.2135</td>
</tr>
<tr>
<td>Cym 12</td>
<td>DQ494846 (CAGA)$_{9}$</td>
<td>R: GATACCTGTAAAGCTGTC</td>
<td>C.s.</td>
<td>174–215</td>
<td>3</td>
<td>0.19/0.20</td>
<td>48</td>
<td>1.0000</td>
<td>–0.0843</td>
</tr>
<tr>
<td>Cym 15</td>
<td>DQ501380 (CT)$_{36}$</td>
<td>R: ATGTCGGCTGTAATGCT</td>
<td>C.s.</td>
<td>129–180</td>
<td>15</td>
<td>0.91/0.90</td>
<td>48</td>
<td>0.0000</td>
<td>–0.0020</td>
</tr>
<tr>
<td>Cym 18</td>
<td>DQ501381 (GT)$_{8}$</td>
<td>R: GATCAAGTGTCTTACGCT</td>
<td>C.s.</td>
<td>124–126</td>
<td>2</td>
<td>0.40/0.18</td>
<td>53</td>
<td>0.0063</td>
<td>0.5447</td>
</tr>
<tr>
<td>Cym 344</td>
<td>DQ494847 (CA)$_{63}$</td>
<td>R: TTTTCTTGTGCTTCGAA</td>
<td>C.s.</td>
<td>171–222</td>
<td>3</td>
<td>0.36/0.32</td>
<td>48</td>
<td>0.0001</td>
<td>0.2059</td>
</tr>
<tr>
<td>Cym 371</td>
<td>DQ501382 (GT)$_{35}$</td>
<td>R: GTGAAAGCCACCTCCATG</td>
<td>C.s.</td>
<td>215–128</td>
<td>1</td>
<td>0.62/0.48</td>
<td>53</td>
<td>0.0059</td>
<td>0.2059</td>
</tr>
<tr>
<td>Cym 8</td>
<td>DQ501383 (GTCT)$_{8}$</td>
<td>R: AGTTGCGGGTCAGTGTAAC</td>
<td>C.s.</td>
<td>283</td>
<td>1</td>
<td>0.91/0.90</td>
<td>48</td>
<td>0.0000</td>
<td>0.5447</td>
</tr>
<tr>
<td>Cym 35</td>
<td>DQ501384 (CA)$_{32}$</td>
<td>R: GATGGATACCTCGCACTG</td>
<td>C.s.</td>
<td>234</td>
<td>1</td>
<td>0.62/0.48</td>
<td>48</td>
<td>0.0000</td>
<td>0.2059</td>
</tr>
<tr>
<td>Cym 41</td>
<td>DQ501385 (CA)$_{18}$</td>
<td>R: TTCAAAGATAAATGCTCC</td>
<td>C.s.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cym 59</td>
<td>DQ501386 (GT)$_{36}$</td>
<td>R: GTTCATTTTCATACTCTCG</td>
<td>C.s.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cym 311</td>
<td>DQ501387 (CA)$_{37}$</td>
<td>R: TTAGCTTGGAGGCGTCGA</td>
<td>C.s.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Repeat motif, primer sequences, species, range of observed alleles ($O$), number of alleles ($A$), observed and expected heterozygosities ($A_{O}$ and $A_{E}$), annealing temperature ($T_{d}$), probability value ($P$) for $\chi^{2}$ test of HWE and GenBank accession no. were provided. Wright’s (1978) fixation index (Fis) as a measure of heterozygote deficiency or excess. C.s. and P.h. = *C. sinense* and *P. hissutissimum*, respectively. A dash indicates no amplification or nonreproductive amplification in the species.

**Literature Cited**


