Development and Characterization of EST-SSR Markers for Ottelia acuminata var. jingxiensis (Hydrocharitaceae)

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Source: Applications in Plant Sciences, 5(11)
Published By: Botanical Society of America
URL: https://doi.org/10.3732/apps.1700083
DEVELOPMENT AND CHARACTERIZATION OF EST-SSR MARKERS FOR *OTTelia ACUMINATA VAR. JINGXIENSIS* (HYDROcharitaceae)\(^1\)

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- **Premise of the study:** Simple sequence repeat (SSR) markers were derived from transcriptomic data for *Ottelia acuminata* (Hydrocharitaceae), a species comprising five endemic and highly endangered varieties in China.
- **Methods and Results:** Sixteen novel SSR markers were developed for *O. acuminata var. jingxiensis*. One to eight alleles per locus were found, with a mean of 2.896. The observed and expected heterozygosity ranged from 0.000 to 1.000 and 0.000 to 0.793, respectively. Interestingly, in cross-varietal amplification, 13 out of the 16 loci were successfully amplified in *O. acuminata var. acuminata*, and 12 amplified in each of the other three varieties of *O. acuminata*.
- **Conclusions:** These newly developed SSR markers will facilitate further study of genetic variation and provide important genetic data needed for appropriate conservation of natural populations of all varieties of *O. acuminata*.

**Key words:** expressed sequence tag–simple sequence repeat (EST-SSR); Hydrocharitaceae; microsatellite; *Ottelia acuminata var. jingxiensis*; transcriptome.

*Ottelia acuminata* (Gagnep.) Dandy (Hydrocharitaceae), a herbaceous perennial, is mainly distributed in the Yunnan–Guizhou Plateau (YGP) and adjacent areas (Chen et al., 2017). Currently, five varieties are recognized under this species based on morphological characters and phylogenetic analysis (Chen et al., 2017), including *O. acuminata var. acuminata*, *O. acuminata var. jingxiensis* H. Q. Wang & S. C. Sun, *O. acuminata var. lunanensis* H. Li, *O. acuminata var. crispa* (Hand.-Mazz.) H. Li, and *O. acuminata var. songmingensis* Z. T. Jiang, H. Li & Z. L. Dao. All of these varieties, with the exception of *O. acuminata var. acuminata*, are each restricted either to a lake, a stream, or a small river (Li, 1981).

*Ottelia acuminata var. jingxiensis*, which can be distinguished from the other varieties by its abundant flowers in the spathe, is distributed in a single river system in Guangxi Province (Wang et al., 1992). This variety is incredibly sensitive to water pollution, and as a result of habitat degradation and human disturbance, most of its previously known natural populations continue to decline and are gradually becoming extinct in many locations (Zhi-Zhong Li, personal observation, 2017). The five varieties of *O. acuminata* have been recorded as endemic and highly endangered species in China (SEPA and IBCAS, 1987). Therefore, investigating the levels and distribution of genetic diversity will be vital for the conservation and management strategies of this species.

Xu et al. (2012) and Lu et al. (2014) reported eight and nine polymorphic microsatellite primers developed from two and three populations of *O. acuminata var. acuminata*, respectively. The two studies applied the Fast Isolation by AFLP of Sequences Containing repeats (FIASCO) technique. However, when we tested these primers, we found low (<50%) amplification efficiency from our tests, in which only eight primers were successfully amplified in *O. acuminata var. jingxiensis*. Hence, more effective simple sequence repeat (SSR) markers are needed for this group. Here, 16 novel SSR markers were developed from transcriptomic analysis of *O. acuminata var. jingxiensis*. Subsequently, marker validation tests were conducted on individuals from three populations of *O. acuminata var. jingxiensis*, and transferability tests were performed on five individuals from each of the other four varieties of *O. acuminata*.

**METHODS AND RESULTS**

Living individuals of *O. acuminata var. jingxiensis* were sampled from Jingxi, Guangxi Province, China, and transplanted in a greenhouse at Wuhan Botanical Garden, Chinese Academy of Sciences. Total RNA was extracted from fresh leaves using RNAiso Plus (TaKaRa Biotechnology Co., Dalian, China) following the manufacturer’s instructions. Quality control and library preparation were performed following the manufacturer’s instructions. Quality control and library preparation were performed following the manufacturer’s instructions.

\(^1\)Manuscript received 2 August 2017; revision accepted 22 September 2017.

The authors thank Ying Zhang and Shi-Xu Huang for their assistance in the laboratory, and Wen Huang for his help in fieldwork. This work received financial support from the Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDPB02) and the National Natural Science Foundation of China (grant no. 31570220).

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doi:10.3732/apps.1700083


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performed following Li et al. (2017). The cDNA library was constructed and sequenced using the Illumina HiSeq X Ten (Illumina, San Diego, California, USA) to produce 150-bp paired-end reads. The generated raw reads were subjected to the stringent filtering process, and a total of 53,865,436 clean reads were generated and assembled de novo into 77,758 contigs (N50 = 1473 bp) based on the optimum length of 20 bp (18–27 bp), annealing temperatures of 55–65 °C (optimal temperature = 60°C), and a product size range of 100–300 bp. Finally, 3794 novel primer pairs were successfully designed for the same SSRs were detected using the perl script MISA (Thiel et al., 2003) with default settings. A total of 6376 SSRs were identified. Among them, trinucleotide repeats (2490, 39.05%) were the most common, followed by dinucleotide repeats (2306, 36.17%), and tetra-, penta-, and hexanucleotide repeats constituted 24.78%. Subsequently, all SSRs were selected for primer design using Primer3 software (Rozen and Skaletsky, 1999). Primer pairs were selected based on the optimum length of 20 bp (18–27 bp), annealing temperatures of 55–65°C (optimal temperature = 60°C), and a product size range of 100–300 bp. Finally, 3794 novel primer pairs were successfully designed for the same...
The 16 SSR markers developed here will facilitate further study on genetic variation in *O. acuminata*. Successful cross-transferability tests of the newly developed markers were conducted among varieties of *O. acuminata*. Therefore, these primers are crucial for further molecular research on *O. acuminata* to provide appropriate genetic information for their effective conservation.

### LITERATURE CITED


### APPENDIX 1. Sampling information of *Ottelia acuminata* used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Population code</th>
<th>N</th>
<th>Locality</th>
<th>Geographic coordinates</th>
<th>Voucher no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. acuminata</em> (Gagnep.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB</td>
<td>15</td>
<td>Debao, Guangxi</td>
<td>22°35’N, 106°16’E</td>
<td>HIB-Otte009</td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>22</td>
<td>Yongfu, Guangxi</td>
<td>25°07’N, 109°44’E</td>
<td>HIB-Otte012</td>
</tr>
<tr>
<td></td>
<td>LZ</td>
<td>5</td>
<td>Luzhai, Guangxi</td>
<td>24°42’N, 109°41’E</td>
<td>HIB-Otte013</td>
</tr>
<tr>
<td></td>
<td>DA</td>
<td>5</td>
<td>Du’an, Guangxi</td>
<td>24°04’N, 108°03’E</td>
<td>HIB-Otte014</td>
</tr>
<tr>
<td><em>O. acuminata</em> var. <em>acuminata</em></td>
<td>HQ</td>
<td>5</td>
<td>Heqing, Yunan</td>
<td>26°24’N, 100°09’E</td>
<td>HIB-Otte003</td>
</tr>
<tr>
<td><em>O. acuminata</em> var. <em>crispa</em> (Hand.-Mazz.)</td>
<td>LGH</td>
<td>5</td>
<td>Luguhu, Yunan</td>
<td>27°40’N, 100°46’E</td>
<td>HIB-Otte011</td>
</tr>
<tr>
<td>H. Li</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. acuminata</em> var. <em>lunanensis</em> H. Li</td>
<td>LN</td>
<td>5</td>
<td>Shilin, Yunnan</td>
<td>24°50’N, 103°27’E</td>
<td>HIB-Otte008</td>
</tr>
<tr>
<td><em>O. acuminata</em> var. <em>songmingensis</em></td>
<td>SM</td>
<td>5</td>
<td>Songming, Yunnan</td>
<td>25°16’N, 102°52’E</td>
<td>HIB-Otte007</td>
</tr>
<tr>
<td>Z. T. Jiang, H. Li &amp; Z. L. Dao</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note:* N = number of individuals.

*Voucher specimens from all populations were deposited in the herbarium of the Wuhan Botanical Garden (HIB), Chinese Academy of Sciences, Wuhan, Hubei Province, China.*