Development of Microsatellite Markers in the Hexaploid Aquatic Macrophyte, Myriophyllum spicatum (Haloragaceae)

Authors: Wu, Zhi-Gang, Yu, Dan, and Xu, Xin-Wei

Source: Applications in Plant Sciences, 1(2)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1200230

Applications in Plant Sciences 2013 1(2): 1200230

PRIMER NOTE

DEVELOPMENT OF MICROSATELLITE MARKERS IN THE HEXAPLOID AQUATIC MACROPHYTE, *MYRIOPHYLLUM SPICATUM* (HALORAGACEAE)[1]

ZHI-GANG WU[2], DAN YU[2], AND XIN-WEI XU[2,3]

[2]National Field Station of Freshwater Ecosystem of Liangzi Lake, College of Life Sciences, Wuhan University, Wuhan 430072, People's Republic of China

- *Premise of the study:* We developed microsatellite primers to investigate genetic diversity and population genetic structure of the cosmopolitan submerged plant *Myriophyllum spicatum*.
- *Methods and Results:* Twenty microsatellite loci were identified in *M. spicatum* using the microsatellite-enriched library method. The numbers of alleles per locus ranged from one to 13, and the expected heterozygosity varied from 0 to 0.873 with a mean of 0.504 in two Chinese populations of *M. spicatum*. All of the loci were also found to be amplifiable in the related species *M. verticillatum* and *M. sibiricum*.
- *Conclusions:* The results indicate that these markers will be significant for studies of population genetic structure and evolutionary history of *M. spicatum* as well as some of its related species.

Key words: Haloragaceae; microsatellite markers; *Myriophyllum spicatum*; polyploid; population genetic structure.

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a perennial submerged macrophyte native to Europe, Asia, and northern Africa (Couch and Nelson, 1985). In North America, *M. spicatum* has been recognized as a noxious invasive plant mainly due to the rapid spread of this species (Reed, 1977; Jacono and Richerson, 2003). *Myriophyllum spicatum* is hexaploid, and the chromosome number ($2n = 6x = 42$) was reported for plants from Europe and North America (Löve, 1961; Aiken et al., 1979), whereas there was no report about the polyploid types of *M. spicatum* because its chromosomes were found to be too small to disclose morphological characteristics for karyotype analysis (Aiken, 1981). There have been numerous studies concerned with the ecology and management of *M. spicatum*, and only a few studies have revealed DNA sequence variation among different individuals (e.g., Moody and Les, 2007). No investigation has been carried out to examine genetic variation in *M. spicatum* at the population level; the evolutionary processes of this species are more likely distinctive due to its occurrence in exclusively aquatic habitats (Barrett et al., 1993). Therefore, we isolated 20 microsatellite markers from *M. spicatum* for use in investigations of genetic variation, population genetic structure, and evolutionary history of this cosmopolitan submerged species.

[1]Manuscript received 8 May 2012; revision accepted 6 August 2012.

The authors thank Song Ge's group for help in molecular techniques. The experiments were partly conducted at the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences. This study was supported by grants from the National Natural Science Foundation of China (30930011 and 31070190).

[3]Author for correspondence: xuxw@whu.edu.cn

doi:10.3732/apps.1200230

METHODS AND RESULTS

Total genomic DNA was extracted from the dried leaves of one individual of *M. spicatum* sampled from the Tai Lake population (Appendix 1) using the DNAsecure Plant Kit (Tiangen Biotech, Beijing, China). A microsatellite-enriched library was developed following the protocol of Glenn and Schable (2005). The genomic DNA was digested into ~500-bp fragments with *Rsa*I and *Xmn*I (New England Biolabs, Ipswich, Massachusetts, USA) and ligated to the SuperSNX24 adapters (F: 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′, R: 5′-pGATTCTGCTAGCTAGGCCTTAAACAAA-3′). The digestion-ligation mixture was hybridized with 3′ biotinylated oligo probes $(AC)_{18}$/$(AG)_{18}$/$(ATG)_{12}$ and captured by Dynabeads M-280 streptavidin (Invitrogen, Dynal AS, Oslo, Norway) for enrichment of simple sequence repeat (SSR) sequences. The products were recovered by PCR amplification with the SuperSNX24 forward primer, ligated into the pEASY-T1 Simple Cloning Vector (Transgen, Beijing, China), and then transformed into competent cells of *E. coli*. Eighty-three positive clones were selected and sequenced with the ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, California, USA). Forty-three clones, or approximately 50% of the positive clones, contained SSRs.

PCR primers were designed for all 43 sequences using the program Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA) and evaluated in 20 individuals from six different populations of *M. spicatum* (Appendix 1). Twenty pairs of primers (Table 1) that showed single and clear bands were chosen and labeled with the fluorescent dyes 6-FAM, HEX, or ROX. Characterization of the SSR loci was estimated in two distant populations in China (Bosten Lake population and Liangzi Lake population; Appendix 1), each with 20 individuals. PCR amplifications were performed in 15 μL total volume containing ~50 ng genomic DNA, 0.33 μM of each primer, and 1× PCR Mix (Tiangen Biotech). Microsatellites were amplified under the following conditions: 5 min initial denaturation at 94°C; 35 cycles of 30 s at 94°C, 30 s at 52–60°C (Table 1), and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR products were analyzed on the ABI 3730XL and genotyping was performed using GeneMapper version 4.0 software (Applied Biosystems).

Because *M. spicatum* is hexaploid, up to six alleles per locus should be expressed in one single plant. Of all 20 loci, however, most showed no more than four alleles per individual (Table 2); no reliable explanation could be provided for this considering that the inheritance pattern of *M. spicatum* was ambiguous. The allele dosage of partial heterozygotes is difficult to identify, thus the presence/absence of the peaks was used to calculate the frequencies for Nei's expected heterozygosity. The locus Myrsp12 showed the highest polymorphism

TABLE 1. Characteristics of 20 microsatellite markers developed in *Myriophyllum spicatum*.

Locus	Primer sequences (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)	Fluorescent dye[a]	GenBank accession no.
Myrsp1	F: GTCAAAGCAGCCACTCGG R: GGCAACAATGCAGCTAACC	$(TCA)_3(TCAGCA)_2(GCA)_3$	179–184	59	6-FAM	JX000192
Myrsp2	F: TTCTACCGCGAAAGACT R: CCATCATTCCTATCAACC	$(AC)_4(TC)_{10}$	340–358	52	6-FAM	JX000193
Myrsp3	F: CACCACTCCTAAACTCACCCTC R: GCTCATCGCCAACTCCTG	$(TA)_7(TG)_{18}$	439–457	60	6-FAM	JX000194
Myrsp4	F: ACTGGCTAATGATATGCTGA R: TCTTTCCACGCCTCTTC	$(TC)_{17}(AC)_9$	253–284	54	ROX	JX000195
Myrsp5	F: GGGAAGCCGACAAGAAA R: CGAAGACGGAGTTATCAAG	$(TC)_{11}$	351–360	55	ROX	JX000196
Myrsp6	F: TAACAAACCGTACATTACAAGC R: TTTCTCTGGGAGCCATAAC	$(TC)_{17}$	145–155	59	ROX	JX000197
Myrsp7	F: AGGACGGAGATAGGATGG R: GAGGGCAAAGGGATGAC	$(TGA)_{10}(TC)_3(TG)_3$	297–307	60	6-FAM	JX000198
Myrsp8	F: GCACCATTAGGAGGAGAAC R: CTGCCGAAGATGAAACG	$(CA)_9$	282–287	58	HEX	JX000199
Myrsp9	F: TCCCCATCTGGTTCGTAT R: GGAAGGTAGCGGAGTGC	$(ATC)_5(TTCATC)_2(TTC)_2$	224–233	58	HEX	JX000200
Myrsp10	F: CTAATCCCAGTCCACGG R: GCTGAAATTGAAGCCTCT	$(TCA)_4(GCA)_5$	268–273	59	HEX	JX000201
Myrsp11	F: ATTCCAATCCCACAGTCT R: TCGGCTCATTAGTCCC	$(GAA)_3(TGC)_6(TGA)_3$	267–269	55	ROX	JX000202
Myrsp12	F: CGCTTCACAAGTATTCTG R: TTCATGGTAGCCGTCA	$(TC)_{18}(AC)_{10}$	349–384	52	HEX	JX000203
Myrsp13	F: GCTTCCATTGCGAAACTT R: CCCAAACACCACCTCATT	$(GCA)_4(TCA)_4(GCA)_3$	450–455	55	ROX	JX000204
Myrsp14	F: TTCCCATCCTTCTCCTG R: CCAAGTAAGTGTCCCAAAC	$(TA)_2(TG)_8(TA)_8(GA)_4$	301–313	58	ROX	JX000205
Myrsp15	F: TCTTTCCACGCCTCTTC R: ACTGGCTAATGATATGCTGA	$(TG)_7(AG)_9$	247–282	56	6-FAM	JX000206
Myrsp16	F: GGCTGCCCTATGCTAA R: ATCCCACTGAAGTCAAACT	$(TG)_2(CA)_8(TA)_6(GA)_6$	339–354	54	ROX	JX000207
Myrsp17	F: CGGAAATACAGTCCAAGGT R: CATGAGACACTAGTAAAGATCGA	$(GT)_2(TG)_9$	320	58	HEX	JX000208
Myrsp18	F: GACGCCAAATCCAACT R: AATGATGTGCCTATACTGAA	$(TCA)_{11}$	331–339	58	6-FAM	JX000209
Myrsp19	F: CTCACCGCCTCGTTAAA R: CGTGTTCATTCCATCATTG	$(TC)_8$	138–148	54	HEX	JX000210
Myrsp20	F: ACCTCTTCTTCCTGTCTACC R: ACTAAGCCACATCTGTCTGA	$(AG)_{13}$	104	57	6-FAM	JX000211

Note: T_a = annealing temperature.
[a]All forward primers were labeled with fluorescent dyes except Myrsp4, Myrsp6, and Myrsp19, in which the labels are on the reverse primers.

with 13 alleles in the Liangzi Lake population, whereas Myrsp17 and Myrsp20 were monomorphic in both populations. The expected heterozygosity ranged from 0 to 0.873 with a mean of 0.407 and 0.601 in the two populations, respectively (Table 2).

Cross-species amplification was conducted in *M. verticillatum* L. (10 individuals, Appendix 1) and *M. sibiricum* Kom. (20 individuals, Appendix 1), both of which are in the same section of *Myriophyllum* as *M. spicatum* (Moody and Les, 2010). All of the loci were amplified successfully in these two related species.

CONCLUSIONS

The polymorphism observed for the microsatellite loci we isolated is high enough to support genetic studies in *M. spicatum*. Cross-species amplification also reveals that these markers are suitable to use in two related species. We conclude that these primers will facilitate the investigation of genetic diversity, population structure, and evolutionary history of *M. spicatum* as well as some of its related species.

LITERATURE CITED

AIKEN, S. G. 1981. A conspectus of *Myriophyllum* (Haloragaceae) in North America. *Brittonia* 33: 57–69.

AIKEN, S. G., P. R. NEWROTH, AND I. WILE. 1979. The biology of Canadian weeds. 34. *Myriophyllum spicatum* L. *Canadian Journal of Plant Science* 59: 201–215.

BARRETT, S. C. H., C. G. ECKERT, AND B. C. HUSBAND. 1993. Evolutionary processes in aquatic plant populations. *Aquatic Botany* 44: 105–145.

COUCH, R., AND E. NELSON. 1985. *Myriophyllum spicatum* in North America. *In* L. W. J. Anderson [ed.], Proceedings of the First International Symposium on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species, 8–18. Aquatic Plant Management Society, Vicksburg, Mississippi, USA.

GLENN, T. C., AND N. A. SCHABLE. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* 395: 202–222.

JACONO, C. C., AND M. M. RICHERSON. 2003. *Myriophyllum spicatum* L. nonindigenous aquatic species. Website: http://nas.er.usgs.gov/taxgroup/plants/docs/my_spica.html [accessed 7 May 2012].

LÖVE, A. 1961. Some notes on *Myriophyllum spicatum. Rhodora* 63: 139–145.

TABLE 2. Results of initial primer screening in two populations of *Myriophyllum spicatum*.

		Bosten Lake		Liangzi Lake	
Locus	A_m	A	H_e	A	H_e
Myrsp1	3	4	0.661	3	0.591
Myrsp2	3	3	0.594	9	0.847
Myrsp3	2	1	0	5	0.417
Myrsp4	4	2	0.496	10	0.782
Myrsp5	5	4	0.703	8	0.817
Myrsp6	3	5	0.568	4	0.681
Myrsp7	2	1	0	5	0.687
Myrsp8	2	2	0.496	2	0.496
Myrsp9	3	1	0	8	0.716
Myrsp10	2	4	0.543	4	0.543
Myrsp11	2	1	0	2	0.466
Myrsp12	6	3	0.667	13	0.873
Myrsp13	3	2	0.496	3	0.631
Myrsp14	4	4	0.543	10	0.773
Myrsp15	4	4	0.543	10	0.784
Myrsp16	4	3	0.665	4	0.689
Myrsp17	1	1	0	1	0
Myrsp18	4	3	0.665	4	0.727
Myrsp19	2	2	0.496	2	0.496
Myrsp20	1	1	0	1	0
Mean	3	2.55	0.407	5.4	0.601

Note: A = number of alleles; A_m = maximum allele number per individual; H_e = expected heterozygosity.

MOODY, M. L., AND D. H. LES. 2007. Geographic distribution and genotypic composition of invasive hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) populations in North America. *Biological Invasions* 9: 559–570.

MOODY, M. L., AND D. H. LES. 2010. Systematics of the aquatic angiosperm genus *Myriophyllum* (Haloragaceae). *Systematic Botany* 35: 121–139.

REED, C. F. 1977. History and distribution of Eurasian watermilfoil in United States and Canada. *Phytologia* 36: 417–436.

APPENDIX 1. Geographic location and voucher information of *Myriophyllum* populations in this study. All voucher specimens are deposited at the Wuhan University Herbarium (WH).

Species	Population	Location	Geographic coordinates	Voucher no.
M. spicatum	FY	Fuyang, Zhejiang	29°59′40″N, 119°41′40″E	*Xu et al., 1051*
M. spicatum	TJ	Tongjiang, Heilongjiang	47°30′06″N, 133°05′10″E	*Xu et al., 201*
M. spicatum	BM	Bomi, Tibet	29°54′58″N, 95°38′05″E	*Xu et al., 2464*
M. spicatum	Tai Lake	Suzhou, Jiangsu	31°13′22″N, 120°26′46″E	*Xu et al., 1017*
M. spicatum	Liangzi Lake	Ezhou, Hubei	30°15′30″N, 114°33′30″E	*Xu et al., 2616*
M. spicatum	Bosten Lake	Bohu, Xinjiang	41°54′24″N, 86°43′53″E	*Xu et al., 2570*
M. verticillatum	Xinkai Lake	Mishan, Heilongjiang	45°20′43″N, 132°22′16″E	*Xu et al., 137*
M. sibiricum	DQ	Deqin, Yunnan	28°30′22″N, 98°54′41″E	*Xu et al., 2450*