Development of polymorphic SSR markers in the razor clam (*Sinonovacula constricta*) and cross-species amplification

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ABSTRACT. Next-generation sequencing provides large-scale sequencing data with relative ease and at a reasonable cost, making it possible to identify a large amount of SSR markers in a timely and cost-effective manner. On the basis of the transcriptome database of *Sinonovacula constricta* obtained by Illumina/Solexa pyrosequencing, 60 polymorphic SSR markers were developed and characterized in 30 individuals. The number of alleles per polymorphic locus ranged from 2 to 7 with an average of 3.75 alleles. The observed and expected heterozygosities varied from 0.050 to 1.000 and from 0.050 to 0.836, respectively. Nineteen loci significantly deviated from Hardy-Weinberg equilibrium ($P < 0.01$) after Bonferroni's correction for multiple tests. In addition, interspecific transferability revealed that 20 polymorphic loci in *Solen linearis* were first characterized in this study. To the best of our knowledge, this is the highest number of SSRs in *S. constricta* and the first report of cross-species amplification. These novel polymorphic SSR markers will be particularly useful for conservation

genetics, evolutionary studies, genetic trait mapping, and marker assisted selection in the species.

Key words: *Sinonovacula constricta*; Expressed sequence tags; Single sequence repeats; Cross-species amplification

INTRODUCTION

The razor clam *Sinonovacula constricta* (Lamarck 1818), a member of benthic bivalve species, lives in the lower-to-mid intertidal zones along the coast of the West Pacific Ocean. Because of its excellent flavor and commercial importance, it has become a popular seafood and is the main cultured economic shellfish, with an annual yield of approximately 700,000 t in China. However, with the fast development of clam aquaculture over the past two decades, the wild stocks of *S. constricta* have dramatically decreased due to over-exploitation, and the genetic diversity of cultured populations has also declined (Wang et al., 2005; Yan et al., 2010). Because it is an economically important fishery species, the significant decline of *S. constricta* stocks has led to attention being placed on present knowledge of genetic conservation and stock improvement for natural resource management and sustainable utilization. Molecular markers are useful tools in conservation genetics and permit the analysis of population structure, genetic diversity, and gene flow (Wan et al., 2004).

Microsatellite markers or simple sequence repeats (SSRs) are one of the most powerful markers in genetic research and are used to determine genetic diversity, for paternity assessment, genome mapping, and in association studies. SSRs have been developed in a wide number of bivalve species, such as Zhikong scallop *Chlamys farreri* (Zhan et al., 2008), Pacific oyster *Crassostrea gigas* (Li et al., 2011), hard clam *Meretrix meretrix* (Wang et al., 2011), and blood clam *Tegillarca granosa* (Dong et al., 2012). Furthermore, expressed sequence tag-derived SSRs (EST-SSRs) can be associated with a function and linked more easily to a phenotypic trait of interest, making them useful for functional diversity studies (Tranbarger et al., 2012). Recently, a large number of ESTs were identified from the *S. constricta* transcriptome using 454 pyrosequencing, providing valuable resources for the efficient characterization of EST-SSR markers (Niu et al., 2013). Although, to date, a total of 70 polymorphic SSRs in *S. constricta* have been isolated and characterized (Niu et al., 2008; Jiang et al., 2010; Liu et al., 2012; Wu et al., 2014; Ma et al., 2015), only 14 EST-SSRs are available, which are not adequate for genetic studies.

In the current study, we report 60 polymorphic EST-SSR in *S. constricta* and their cross-species transferability. These novel SSR markers will be particularly useful for conservation genetics, evolutionary studies, genetic trait mapping, and marker-assisted selection in this species.

MATERIAL AND METHODS

Genomic DNA was extracted from the adductor muscle of 30 adult individuals of *S. constricta*, collected from Ninghai Zhejiang Province, China, using the standard proteinase digestion and phenol-chloroform extraction method (Sambrook and Russell, 2002). The quality and concentration of total DNA were examined on 1.2% agarose gel electrophoresis and by the OD_{260}/OD_{280} ratio using a NanoVue UV/visible spectrophotometer (GE Healthcare UK Limited, UK). DNA templates were diluted with ultrapure water to obtain a 100 ng/μL final concentration.

Previously, we sequenced a comprehensive transcriptome of *S. constricta* using the Illumina HiSeq 2000 platform. Putative SSR markers were screened using the SSRHNUTER program (Li and Wan, 2005). Primers flanking microsatellites were designed by the Primer Premier 5.0 software.

PCR amplifications were performed in a 20-μL reaction mixture containing 0.5 U *Taq* DNA polymerase (Takara), 1X PCR buffer, 0.2 mM dNTP mix, 2.5 mM $MgCl_2$, 0.25 μM each forward and reverse primers, and 100 ng template DNA. The PCR was carried out in a Mastercycler Pro S thermal cycler under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, the primer-specific annealing temperature (Table 1) for 45 s, and extension at 72°C for 45 s with a final extension at 72°C for 7 min. PCR products were separated by electrophoresis on 8% nondenaturing polyacrylamide gel and visualized by ethidium bromide staining under UV light. Allele sizes were identified by comparing bands with a 20-bp DNA ladder (MBI Fermentas, USA). The characterized polymorphic loci were examined for cross-species amplification in *Solen linearis* from Zhoushan City, Zhejiang Province, China, to assess their transferability.

All polymorphic SSR-containing unigenes were searched against the NCBI non-redundant (Nr) protein database and Swiss-Prot database using BLASTx with an e-value of 1e-5. The number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content (PIC) were calculated using the CERVUS 3.0 program (Kalinowski et al., 2007). The P value for Hardy-Weinberg equilibrium (HWE) was estimated using Popgene32 (Yeh et al., 2000). All significant levels were adjusted by the sequential Bonferroni's correction for multiple tests (Rice, 1989).

RESULTS AND DISCUSSION

Based on the 225 SSR-containing unigenes, 124 primer pairs were successfully designed and used to validate the amplification. After optimization of annealing temperature, 84 primer pairs (67.74%) cleanly and consistently amplified the expected products. Of the 84 primer pairs, 60 SSR loci were found to be polymorphic in the population of *S. constricta* (Table 1), resulting in a polymorphic percentage of 71.43%, which was similar to that reported by Jiang et al. (2010) (70.00%) and higher than that reported by Liu et al. (2012) (48.28%). The N_A per polymorphic locus ranged from 2 to 7 with an average of 3.75 alleles, which was similar to previous results obtained from EST-SSRs (Liu et al., 2012). The H_O ranged from 0.050 to 1.000, while the H_E varied from 0.050 to 0.836 (Table 1). The mean PIC was 0.439, ranging from 0.048 to 0.791. Furthermore, 19 highly polymorphic loci (PIC ≥ 0.5) and 32 moderate polymorphic loci (0.25 < PIC < 0.5) were found to be suitable for population genetic analyses. In addition, among the 60 polymorphic loci, 27 (45%) identified from annotated genes were expected to be especially valuable for use in functional diversity studies. After Bonferroni's correction for multiple tests, 19 loci were found to significantly depart from HWE (P < 0.01).

EST-SSR markers possess higher transferability due to their conservation characteristics when compared with genomic SSRs (Zeng et al., 2010). After cross-species amplification, 20 reliable SSRs could be amplified successfully and displayed polymorphisms in *S. linearis*, resulting in a transferability rate of 33.33%. The number of alleles of 20 loci in *S. linearis* ranged from 2 to 7

Table 1. Characteristics of 60 polymorphic EST-SSRs for the razor clam (*Sinonovacula constricta*).

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Size (bp)	N_A	H_O/H_E	PIC	P-HWE	Functional annotations *e- value
comp149939_c0	F: AAATGATGTCGTTTGGTG R: CTGGGTTCTATGCTCAAA	$(AC)_8$	53.4	98-125	4	0.800/0.585	0.492	0.000000*	-
comp146001_c0	F: CTACTATTTCCGTATGTG R: AAGCGTACTACAAGTTAA	$(AT)_7$	53.4	160-183	3	0.400/0.337	0.289	0.783697	-
comp131694_c0	F: ATGTCAATCGGTGAAGGAG R: CTATGCTCAGGGATTTGTG	$(GAG)_5$	56.1	180-202	2	0.850/0.501	0.374	0.001394	Transcription factor AP-1
comp150570_c0	F: GTGTTCAGCAATCAGAGTC R: AAAGTATCTGTTAAGCCAT	$(TA)_6$	56.1	243-268	3	0.650/0.489	0.413	0.238446	Uncharacterized protein LOC101854894
comp145914_c0	F: CCTCTTGTTTTCCTTTTC R: ACTGTGACCAAGTCGTTT	$(TG)_6$	56.1	290-310	3	0.400/0.347	0.311	0.783697	-
comp147130_c0	F: TGATGTCTAACACGACCAA R: GCACCCTACCTCAATAAAC	$(ACC)_5$	58.7	180-198	2	0.350/0.296	0.247	0.384683	-
comp145652_c1	F: CTTCCCTCTTAGAAAGCATC R: ATAGCGGTGTAAAACCAGTC	$(AC)_6$	58.7	140-150	2	0.650/0.450	0.342	0.039898	Nucleolysin TIA-1-like isoform X7
comp150397_c0	F: ATGCCTCCTACATCAGTC R: GTGTTGGTAGTCTGGGTC	$(AAC)_6$	58.7	210-220	2	0.050/0.050	0.048	1.000000	Hypothetical protein DDB_G0286467
comp144588_c0	F:CCACATTGCTCACTGATTAC R:ATTTGTATGAGGGGTGATTT	$(TA)_7$	58.7	205-250	4	0.950/0.560	0.444	0.000000*	-
comp145928_c0	F: ACTGAACACTTGGTCTCCT R: AAGAGCACATCATTTGGTT	$(AT)_6$	61.1	158-208	3	0.900/0.530	0.406	0.005627	-
comp137074_c0	F: TCTACACGGCAGTTGAGG R: AATCCGAGCACAGCATAG	$(CTT)_5$	58.7	130-140	2	0.150/0.142	0.129	0.769866	Proliferation-associated protein 2G4
comp152107_c0	F: GGGCAATCCTTCCACAGT R: TTACAAGCGCGCTACACG	$(CTA)_5$	58.7	198-230	3	0.100/0.099	0.094	0.998828	Glutathione S-transferase A
comp154232_c4	F: TTCCCAGCAAGATGGATAGT R: TTCATCTTTGGAAACACCCT	$(AT)_6$	58.7	240-250	2	0.150/0.142	0.129	0.769866	Hypothetical protein CGI_10025513
comp145529_c0	F: CCACCTCCTCGCCTGTAA R: GCAAGGTACGACCAAAGC	$(CCA)_6$	63.1	160-200	2	0.950/0.512	0.374	0.000084*	Probable splicing factor, arginine/serine-rich 6
comp151831_c0	F: CCCTTACCTCCTTTATTGT R: ACTCTTTAATCCAGCCAAG	$(TA)_6$	58.7	150-180	4	0.150/0.146	0.139	0.999987	Sialic acid binding lectin
comp154472_c0	F: ATTACATCACGGAGTTGC R: ATCAATGACACGGCAGAC	$(ATT)_5$	61.1	165-175	2	0.050/0.050	0.048	1.000000	Protein Star
comp153677_c1	F: CCGACATTACGATATTCAG R: GAGGCTTGAAGTACATTTT	$(AAC)_5$	56.1	190-240	3	0.950/0.627	0.543	0.001455	-
comp154079_c0-1	F: TCCCGAGATTTGCGATAC R: TAATGGGGCAGAATGAAA	$(AC)_9$	56.1	145-175	5	0.950/0.639	0.551	0.000322*	Protocadherin Fat 4-like
comp154079_c0-2	F: TAAGGGACTTTCTTGGAAT R: TATCGCAGGAATAGTTGTA	$(TA)_6$	56.1	280-310	2	0.850/0.501	0.369	0.001394	Protocadherin Fat 4-like
comp155403_c0	F: AAAAGTGCCGAGCCTGAT R: GGGCCAGTGGTATGTTGC	$(AC)_9$	64.5	330-360	4	1.000/0.753	0.685	0.000000*	-
comp153848_c0	F: GAATAGCCATTATGTAACCC R: AGACAGAAAAGGACAAACTC	$(AT)_8$	56.1	90-115	5	0.750/0.600	0.528	0.000641*	-
comp153651_c0	F: CACGAGAATGTAAGGGTA R: CGTCAACTTTATTTTGCT	$(GT)_9$	53.4	105-140	5	0.600/0.782	0.723	0.000000*	-
comp153486_c0	F: AGAGCGTGCTTCGTGGTG R: ATGGGAGGGTTCGGGAGA	$(CAG)_7$	64.5	280-330	4	0.550/0.701	0.626	0.071736	Nuclear receptor subfamily 0 group B member 1
comp153084_c0	F: TTTCCCTATGCTGTGCTA R: GTGTCTGTCTGACCTGCT	$(CAT)_5$	56.1	208-230	3	0.800/0.528	0.424	0.040917	Pleckstrin-like protein domain-containing family F member 2
comp151043_c0	F: GAAAGGATGTTACCACCAC R: GTTCAATCATGCTTTGGAT	$(ATC)_5$	63.1	290-315	2	0.600/0.513	0.375	0.435383	Ras-related protein Rab-1A
comp153741_c0	F: ATACTCAAAATTCTGCTGGGACC R: TAGAATGTGGTGAGGATTTAGGG	$(AT)_6$	56.1	238-265	5	1.000/0.722	0.651	0.003205	Elongation factor Ts
comp153647_c0	F: TGGGGACATACATACTCTA R: GAAAACTTTACTTCCTTGA	$(TA)_7$	50.8	80-105	4	0.400/0.414	0.366	0.001272	Tyrosine-protein phosphatase non-receptor type 61F
comp153643_c1	F: GCTCAAGAACTGACATTATTAT R: ACACTTCCATCTGTACATTATC	$(TA)_7$	58.7	110-130	4	1.000/0.605	0.504	0.000000*	-
comp153526_c0	F: GGCACCTCAACAATCTGAAC R: ATCCAAGAAGCCCTCTAAAA	$(TGT)_5$	56.1	140-165	3	0.400/0.337	0.289	0.783697	Regulator of G-protein signaling 12
comp155384_c0	F: AATGCTTGGACGTTGTTTAT R: TTCTCCCTATTTGAAACTGA	$(AT)_6$	53.4	140-180	3	0.700/0.497	0.406	0.149810	-
comp153621_c1	F: TCATAAGGAGGATTTCGT R: TACAACCCCACTTCATTT	$(TG)_6$	56.1	120-140	4	0.950/0.560	0.444	0.000000*	-
comp147145_c0	F: ATGCTTCATAGAAAAGTCAGT	$(TG)_7$	61.1	240-270	3	0.800/0.569	0.491	0.040917	Hypothetical protein CGI_10025397

Continued on next page

Table 1. Continued.

	R: AACATCAACAGCTTGCTAATA								
comp144354_c0	F: GTGTTGCTCTTTTCATAC R: CTATGTGGAAAGGAAGAT	$(AC)_6$	56.1	190-220	4	0.600/0.481	0.428	0.768076	3-mercaptopyruvate sulfurtransferase-like
comp143020_c0	F: TTCTGGTGCTGAATCTTATC R: TCAACCCACTTACATTACTT	$(TA)_8$	56.1	190-210	4	0.250/0.355	0.329	0.000046*	-
comp140441_c2	F: AACAAAGACATATCGGGAAAA R: AAATACAATCAAGAGGAAAGC	$(TA)_7$	58.7	210-228	3	0.500/0.472	0.410	0.060376	-
comp154150_c0	F: AGTGTAAGAATAAGCCAGTG R: TGATGGAGTTAAATCGTAGT	$(ATT)_5$	58.7	230-245	4	0.150/0.481	0.428	0.000111*	Acidic leucine-rich nuclear phosphoprotein 32 family member A
comp151588_c0	F: TGCCTTGACATACTTGAT R: GTGTCCAGGAATTGTCTC	$(AC)_6$	53.4	210-228	3	0.900/0.530	0.406	0.005627	-
comp153401_c1	F: GTGAACAGTCTGGTGGAT R: TCGAGGCAATGATAGTTT	$(TTG)_5$	56.1	260-290	4	1.000/0.665	0.579	0.004164	-
comp154015_c0	F: AGGCAAACTTGTTCATCAT R: CAAACCTAATGGCATTGTT	$(TTG)_5$	56.1	200-220	3	0.650/0.537	0.455	0.019436	Cisplatin resistance-associated overexpressed protein
comp152191_c0	F: AATATCGGTTGTGGTCTA R: CATTGTAACATGCCTGAA	$(TTG)_5$	45.3	120-135	3	0.250/0.601	0.506	0.005119	-
comp153157_c3	F: CCAAGCAAGTTAAGAGTA R: ATGTCTGGCTTAGATGAG	$(TA)_7$	45.3	180-208	3	1.000/0.559	0.441	0.000273*	-
comp152312_c0	F: CGACGAAGTTACCTGAGAAGA R: GAACACTCCGACAAAGACCTA	$(AT)_6$	63.1	270-295	4	0.550/0.545	0.452	0.000000*	-
comp155173_c1	F: AAGAAATGACATCTGCTA R: ACAGTGTAGGGTTCAGTG	$(AT)_7$	53.4	185-220	5	0.850/0.705	0.644	0.001209	Glycine-rich protein
comp153794_c0	F: TGTTGTAATGCCTTGTTG R: GTGAGAAAGACCTTGGAG	$(TC)_8$	50.8	150-178	5	0.250/0.572	0.497	0.005781	-
comp152512_c0	F: ATTGTAGCCAAAATAGATGAC R: AAGCAAACTATACCTATCAGA	$(TGG)_5$	45.3	278-330	4	0.850/0.647	0.575	0.000000*	Similar to thiopurine *S*-methyltransferase
comp140874_c0	F: TTCAACAAGCGTCATAACTCTGGT R: TGGATTTTCCTGGTCAATAACTTT	$(AT)_6$	58.7	260-295	5	0.550/0.533	0.471	0.884628	-
comp145632_c1	F: ATTCTCGCTCCCTACTGT R: CTTGCGTTCATCTTTGTT	$(TGA)_7$	58.7	285-320	4	0.350/0.453	0.406	0.000143*	Uncharacterized protein LOC101237262
comp150866_c0	F: GGGAACAACACTATTTCG R: GATGCTACAATAGTGGCTCA	$(AC)_6$	58.7	280-315	4	0.900/0.585	0.492	0.050131	-
comp149438_c0	F: ATGATGACGACGATGACAAGAGC R: CTTTAATGCGGCAGGATTAGTGT	$(ATG)_5$	64.5	165-185	3	0.300/0.268	0.238	0.917126	-
comp130865_c0	F: GCCAATGTATCCTCACCCA R: TCAGGGAAGTTCTTGTGCC	$(AGA)_8$	64.5	280-305	5	0.050/0.430	0.397	0.000000*	Calponin-like protein
comp143811_c0	F: TGTCAAAAGTCCGCTCCC R: AAGCCAGGACCATCGTTA	$(TA)_6$	64.5	145-185	7	1.000/0.823	0.776	0.000000*	-
comp148954_c0	F: ATCTTCAGCCCCAGTTGC R: CAAAACAATGCCCCTCTT	$(AC)_7$	58.7	260-290	6	0.350/0.636	0.586	0.003788	-
comp154189_c0	F: TCGTCCATGTATGTTGGT R: CTTCACAGCGTGCCTAAT	$(TA)_8$	58.7	300-325	5	0.450/0.719	0.656	0.022402	-
comp154402_c0	F: ATGAACGAAGCCATCTAC R: TATACATACACCCTGTGC	$(TTG)_5$	58.7	285-325	5	0.450/0.491	0.448	0.920595	-
comp154517_c0	F: TTATGGATGTTTCCTCACCT R: ACATTTTAGGAGTTGCTTTA	$(AT)_8$	53.4	230-265	5	0.850/0.681	0.603	0.000000*	-
comp154118_c1	F: GAAGAAAACCATTGTCCACAGTAA R: GAAGAAAACCATTGTCCACAGTAA	$(TA)_6$	63.1	220-265	5	0.750/0.731	0.667	0.000089*	-
comp154403_c0	F: GTACCAGATAACACCCAT R: CAAGGACAAACGCTCTAT	$(AC)_8$	58.7	205-258	6	1.000/0.817	0.767	0.002057	-
comp153522_c0	F: AATAGAGTTTGGCTACCG R: ATCCAAAGAAGACCTCAA	$(CTG)_8$	53.4	165-210	7	0.950/0.836	0.791	0.012845	Phospholipid scramblase 2
comp148706_c1	F: CTATACGAGCCTCGCTGCACAATT R: AGGATGACATAGAAGAGG	$(AAC)_5$	64.5	270-310	4	0.550/0.450	0.401	0.860108	-
comp149921_c0	F: CTCCAGGGACTCTAAAAGAAT R: CCATCTATAAGACTTCCAAAA	$(AAT)_5$	58.7	200-210	3	0.250/0.232	0.214	0.956353	-
Mean	-	-	-	-	3.75	0.607/0.507	0.439		

Ta = annealing temperature; N_A = number of alleles; H_O/H_E = observed heterozygosity/expected heterozygosity; PIC = polymorphic information content; P-HWE = P value of Hardy-Weinberg equilibrium (*show the significantly departed from Hardy-Weinberg equilibrium). [a]The SSR-containing unigenes were annotated using BLASTx against Nr or Swiss-Prot database with an e-value cutoff of le-5.

with an average of 3.35 alleles, which was slightly higher than the average number of alleles in *S. constricta*. From the genetic parameters of PIC, H_O, and H_E, we found that the genetic diversity and heterozygosity of *S. constricta* was higher than that of *S. linearis* (Table 2).

Table 2. Cross-species amplification of 20 EST-SSRs for *Sinonovacula constricta* and *Solen linearis*.

Locus	*S. constricta* (N = 30)					*S. linearis* (N = 30)				
	N_A	H_O	H_E	PIC	P-HWE	N_A	H_O	H_E	PIC	P-HWE
comp147130_c0	2	0.350	0.296	0.247	0.384683	4	0.222	0.386	0.351	0.015161
comp144354_c0	4	0.600	0.481	0.428	0.768076	2	0.833	0.500	0.368	0.003551
comp152191_c0	3	0.250	0.601	0.506	0.005119	2	0.833	0.513	0.374	0.006319
comp153643_c1	4	1.000	0.605	0.504	0.000000	3	0.611	0.465	0.389	0.373985
comp153647_c0	4	0.400	0.414	0.366	0.001272	3	0.389	0.338	0.300	0.830418
comp153794_c0	5	0.250	0.572	0.497	0.005781	7	0.778	0.722	0.677	0.903583
comp153157_c3	3	1.000	0.559	0.441	0.000273	4	0.222	0.303	0.280	0.083754
comp153486_c0	4	0.550	0.701	0.626	0.071736	2	0.944	0.513	0.374	0.000236*
comp153848_c0	5	0.750	0.600	0.528	0.000641	2	0.333	0.413	0.321	0.394021
comp154472_c0	2	0.050	0.050	0.048	1.000000	2	0.556	0.508	0.372	0.682107
comp145652_c1	2	0.650	0.450	0.342	0.039898	3	0.556	0.446	0.386	0.502284
comp153401_c1	4	1.000	0.665	0.579	0.004164	4	0.389	0.344	0.314	0.989777
comp154015_c0	3	0.650	0.537	0.455	0.019436	2	0.889	0.514	0.375	0.001473*
comp153526_c0	3	0.400	0.337	0.289	0.783697	3	0.111	0.110	0.104	0.998610
comp145914_c0	3	0.400	0.347	0.311	0.783697	5	0.222	0.464	0.421	0.007320
comp154079_c0-2	2	0.850	0.501	0.369	0.001394	3	0.222	0.208	0.190	0.976673
comp153677_c1	3	0.950	0.627	0.543	0.001455	2	0.667	0.457	0.346	0.043775
comp155403_c0	4	1.000	0.753	0.685	0.000000	4	0.722	0.618	0.581	0.105114
comp131694_c0	2	0.850	0.501	0.374	0.001394	5	0.556	0.451	0.396	0.992839
comp155529_c0	2	0.950	0.512	0.374	0.000084	5	0.611	0.735	0.684	0.211279
Mean	3.200	0.645	0.505	0.426	-	3.350	0.533	0.450	0.380	-

N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; P-HWE = P value of Hardy-Weinberg equilibrium.

In summary, we report the highest number of EST-SSRs in *S. constricta* to date. All of the SSR primers in this study will be useful for conservation genetics, comparative mapping, evolutionary biology, and molecular breeding in the species.

Conflicts of interest

The authors declare no conflict of interest.

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