as these are not considered as SSRs due to their presence at 3'-end of mRNA/cDNA sequences.

Primer designing for SSRs

A pair of primer flanking each SSR was designed using FastPCR software available at www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi, which takes input according to user-defined conditions and pick primers according to these specified parameters. Default parameters of the FastPCR, viz, the optimum primer size of 20.0 (the range was 18–28), the optimum annealing temperature of 60.0 (the range was 57.0–63.0), and the range of% GC content of 44–60, were selected for primer designing.

Detection of SSR positions with respect to open reading frames

Open reading frames (ORFs) are predicted for all the SSR-containing sequences using ORF finder available at NCBI (http://www.ncbi.nl m.nih.gov/gorf/gorf.html) using standard genetic code. Sequence fragments with maximum length uninterrupted by stop codon were taken as the primary encoding segment (ORF) of the query sequences. In all the predicted ORFs, the relative positions of SSRs were detected, that is, whether the SSR was present within the ORF, in the 5' UTR untranslated region (UTR) or in the 3' UTR

RESULTS

Screening of *Gentianaceae* sequences for SSRs

In the present study, 4698 nucleotide sequences of *Gentianaceae* available at NCBI (http://www.ncbi.nlm.nih.gov) were searched for SSRs with a minimum length of 18 bp. A total of 545 SSRs were detected from 2889 kb of data mined, excluding poly A and poly T. Depending upon the length of the repeat unit itself (1–6 bp), the lengths of the identified SSRs varied from 14 to 48 bp, respectively.

Frequencies of classified repeat types of *Gentianaceae*

From a number of 4698 sequences screened, only a subset of 461 sequences contained 545 SSRs, suggesting that merely 9.83% of sequences contained SSRs. The frequencies of SSRs with mono-, di-, tri-, tetra-, and hexanucleotide repeat units showed the frequent repeat type within the nucleotide sequences of Gentiana family that were found to be in mononucleotide (84.58%) followed by dinucleotide repeats (18.16%), trinucleotide (2.75%), and hexanucleotide (0.65%), respectively [Figure 1]. Whereas, no tetranucleotide and pentanucleotide repeat was detected during the analysis.

The observed frequency of different repeat types comprising the SSRs is presented in Figure 2a–d and summarized in Table 1. SSRs were comprised of four different types of mononucleotide (A,T, C, and G), nine different types of dinucleotide (CA)n, (TG)n, (AC)n, (GA)n, (CT)n, (TA)n, (AT)n, (GC)n, (TC)n, (AG)n, (GT)n repeats, seven different types of trinucleotide (GAG)n, (ATG)n, (CTT)n, (TTA)n, (CAA)n, (AAC)n, (ACA)n repeats, and two types of hexanucleotide (CCACAC)n, (GGTCAA)n repeats.

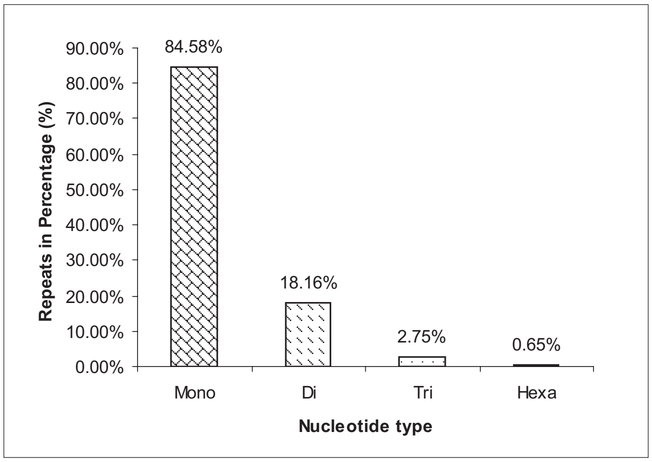


Figure 1: Frequency distribution of different repeat types identified in nucleotide sequences of *Gentianaceae*

Designing of primers for SSRs

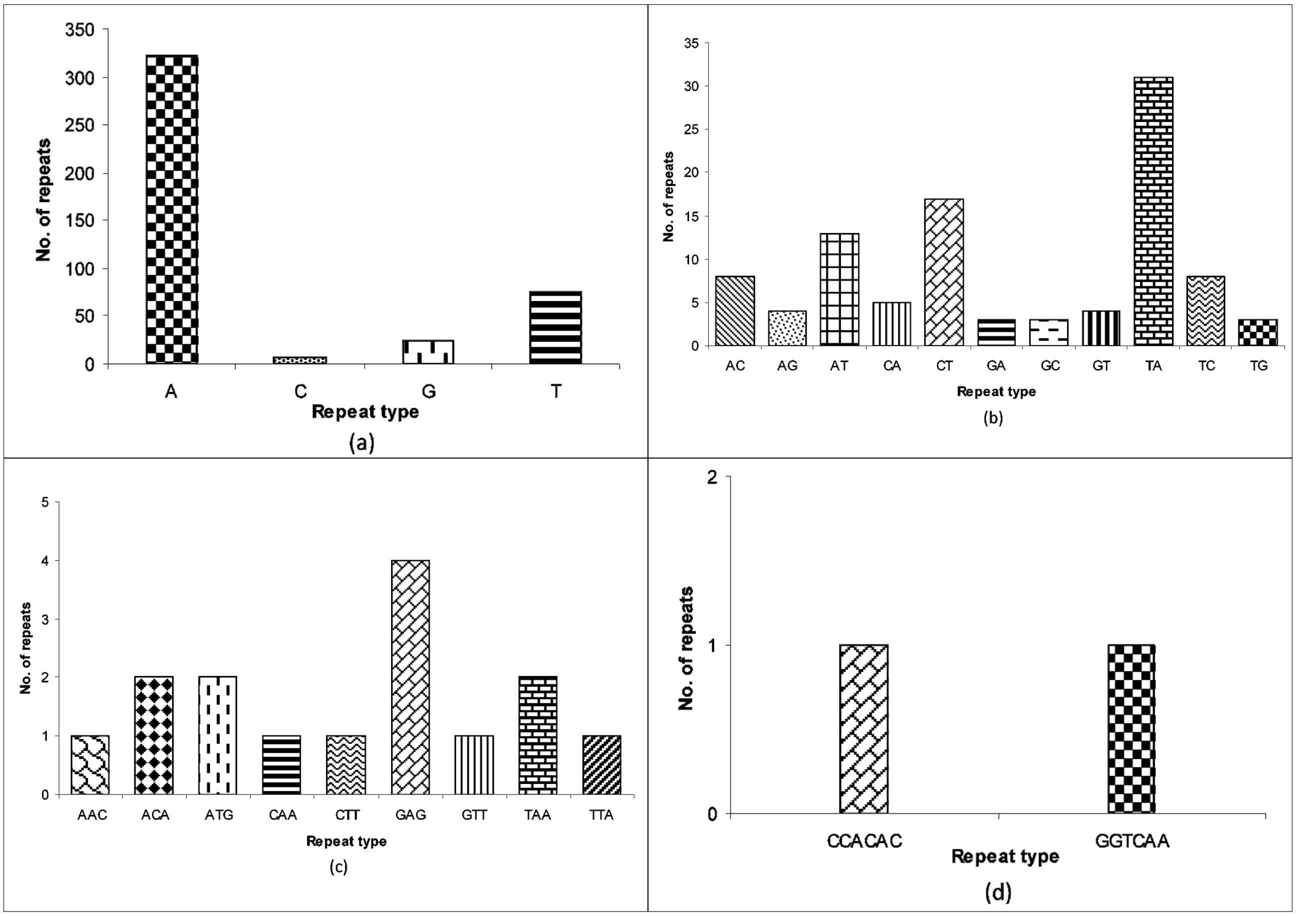
Out of 545 SSRs detected, the primers could be designed only for 169 (31%) SSRs and the rest 376 (69%) sequences did not produce any acceptable primers. These 169 SSRs for which primers were designed include 133 mono-, 29 di-, 7 tri-, and no hexanucleotide repeats. The details of the accession numbers of nucleotide sequences of Gentiana, repeat motif of SSRs for which primer were designed, primer sequences, GC%, product size, and annealing temperature are given in Table 2.

Prediction of ORF in SSR-containing sequences

An attempt was made to predict the ORFs in SSR-containing sequences using ORF finder. Out of the 545 SSRs identified, the positions of 359 SSRs with respect to ORF were determined, while for the remaining 186 SSR-containing sequences, no ORF were predicted. Of these 359 SSRs, a large number of 161 (44.84%) were present in the 5' untranslated region, 129 (35.93%) SSRs occurred within ORF, and the remaining 69 (19.22%) occurred in the 3' untranslated region.

DISCUSSION

In the present study, a large number of nucleotide sequences (4698) of Gentiana retrieved from NCBI were mined for SSRs. In the sequences that were mined the SSRs were characterized, and a subset of these SSRs was



(a)

(b)

(c)

(d)

Figure 2: Frequency distribution of (a) mono-, (b) di-, (c) tri-, and (d) hexanucleotide repeat motifs in the genome of *Gentianaceae*

Table 1: Summary of in silico mining of Nucleotide sequences of *Gentianaceae*

Parameters	Values
Total number of sequences searched	4698
Total number of SSRs after removing poly A and poly T	545
Total size of examined sequences (bp)	2289303
Total number of sequences containing single SSRs	429
Number of sequences containing two SSRs	99
Number of sequences containing three SSRs	15
Number of sequences containing six SSRs	2
Number of sequences containing more than one SSR	57
Number of SSRs present in compound formation	47
Repeat type	
Mononucleotide	429 (84.58)
Dinucleotide	99 (18.16)
Trinucleotide	15(2.75)
Hexanucleotide	2 (0.65)

Data in parentheses is the percentage value of the repeat

used for designing the markers. A total of 545 SSRs was detected and this was in accordance to the findings of[32] who reported that the abundance of different repeats varied broadly depending upon the species.

Microsatellites or SSRs are stretches of DNA containing tandem repeats of di-, tri-, tetra-, and above nucleotide units ubiquitously distributed throughout the eukaryotic genome. They are found to be abundant in plant genomes and are thought to be the major sources of genetic variation in quantitative traits. The abundance of the different repeat motifs (1–6 bp) in the SSRs as detected in Gentiana family during the present study was variable so that the SSRs with different repeat motifs were not evenly distributed. The SSRs with dinucleotide repeats (18.16%) were abundant. This is in agreement with the results of earlier studies on Arabidopsis in which the dinucleotide repeats were also found to be abundant,[33] perhaps because the genomic sequences of this species may include SSRs in noncoding regions too. The smaller repeat motifs were found to be predominant among SSRs identified and as the length of repeat unit increases, their occurrence decreases. We excluded poly A and poly T repeats due to which their number is under-represented. The abundance of trinucleotide SSRs may be attributed to the absence of frame shift mutations due to variation in trinucleotide repeats.[34]

Molecular genetic markers can be used to examine a group of individuals or populations to estimate various diversity measures and genetic distances, intergenetic structure and

Table 2: Synthesis of primer

Accession number	Motif	Forward Primer		Reverse primer		Annealing temperature (C)	Product size (bp)
		Primer	GC %	Primer	GC %		
gi|261865195|gb|GQ864096.1|	(TA)7(AT)7	GGGCAGAACTCAACGGAAGC	59.1	GAGGCTAAAGGGTCGGA	58.8	57.9	709
gi|169798749|gb|EU370947.1|	(A)13	CGAGGCTGTAAAGTGCGTGC	60	GACGGTGAGAGGCTGTGTATG	57.1	56.85	415
gi|24494572|gb|EF371451.1	(TG)17	GAGGCTGTAAAGTGCGTC	50	GACGGTTAGAGGCTGTGTAGG	54.5	54.25	414
gi|124494568|gb|ef371447.1|	(AG)7(TG)8 (GA)12	GTCTGAGTGAGGGAGCCATC	60	GTGATGCTGTGTGTCCCAAGAG	54.5	56.8	80
gi|112293600|gb|dq822583.1|	(CA)29(GA)21(AG)6	GGGATGGCACTCACCTACAGC	61.9	GGCATAGTCTGAGTTCCCAC	55	54.45	123
gi|112293594|gb|dq822577.1|	(CT)21	GCGTTCATCCGACTGCCGAG	65	GCAGAGCATAGTGGACGC	61.1	62	
gi|156787489|gb|ef569230.2	(A)21	CTGTGTGATGCTGAGGTTGC	55	GGCTGAACCACGGGACAACC	65	59	656
gi|89199712|gb|dq398766.1|	(A)16	GTAGATGTTCAGAGGACG	50	GGTTGACACTTTACAGCACG	50	57	461
gi|89199711|gb|dq398765.1|	(A)10	GCGATGTGTTCAAGGACCG	55	GTGAACGCCTATCCAGTG	55.6	59	760
gi|89199709|gb|dq398763.1|	(A)16	CGCTGTTCCGCTCACGCTTC	65	GTTGACACTTTACAGCCCCT	50	63	676
gi|89199708|gb|dq398762.1|	(A)16	GACGCTGTAAAGTGATAG	44.4	GCGTTAGGCTGACTTCGTG	57.9	56	409
gi|89199707|gb|dq398761.1|	(A)12	GTCGCTGAGTAGTGCCAAG	57.9	GGCGGTTAGGGGCTGACACG	70	64	349
gi|89199706|gb|dq398760.1|	(A)12	GCTGCTGTAAAGTGCCAACG	55	GAGGCACGATTAGGGGCTGAC	61.9	65	345
gi|89199705|gb|dq398759.1	(A)12	GAGCCTGTAAAGTGCCAAC	52.6	GTGTCGTGTAGAGCCTGAACCG	59.1	64	414
gi|89199704|gb|dq398758.1|	(A)11	GACGCTGTAAAGTGCCAAC	52.6	GTAGGCGTAACTCTGACAC	52.6	58	806
gi|89199703|gb|dq398757.1|	(A)11	GAGGTGCTGTAGAGTGCCATC	57.1	CGGCGGATAGAGATAGAG	56.6	61	228
gi|89199702|gb|dq398756.1|	(A)11	GAGTCTGTAGGTGCCGAACG	60	GCGAGATTAGAGCGTGAACAG	52.4	64	539
gi|89199701|gb|dq398755.1|	(A)11	GTCGCTGTAAAGTGCTAAC	47.4	GTCATCAAGTCGCACAGGC	57.9	58	313
gi|89199700|gb|dq398754.1|	(A)12	CTGTTCAAAGGACCCGTGC	57.9	GAGCCTTTTGTCGTCGGTGG	60	56.35	346
gi|89199699|gb|dq398753.1|	(A)12	GGCGGCTGTAAGTCCATCC	63.2	GCTTGCTAACGGATTCTGCG	55	58	415
gi|89199698|gb|dq398752.1|	(A)11	GGAGAGTAAGTGCCAACC	55.6	GAGCATTGTCCGAACGAGCG	60	56.55	409
gi|89199697|gb|dq398751.1|	(A)11	GGAGGCTGGCTAAAGTGCTAC	57.1	GAGCCATTGCGAACAGTG	55.6	56.55	409
gi|89199696|gb|dq398750.1|	(A)15	CAATGTTCAAGCGATGCCGTC	52.4	CTATGGTCAGGAACGGTGG	57.9	57.05	349
gi|89199695|gb|dq398749.1|	(A)13	CATCAGAGTTCAAGGACCG	52.6	GATACCTTGTCTACGAACGG	50	57.35	345
gi|89199694|gb|dq398748.1|	(A)21	GGAGGTCTGCTAAGTGCCAAC	57.1	GCACCTTGTCGGAACGGCGG	70	58	419
gi|89199693|gb|dq398747.1|	(A)15	CATCGTGTTCAAGGACCG	55.6	GAACCTCTGTCGGAACGG	61.1	57.05	349
gi|89199692|gb|dq398746.1|	(A)12	GTGCTGTATCAAGGACCGC	57.9	GAGCCTTGTGAACGGTGG	61.1	57.05	349
gi|89199690|gb|dq398744.1|	(A)12	GGAGGTCTAAGTGCCAAC	55.6	GGTCGTGCGGTTAGGTGG	66.7	59.75	141
gi|89199689|gb|dq398743.1|	(A)14	GGACGGTGTAAGTGCCAAC	57.9	GAAGGTCCACGGTTAGGT	55.6	59.75	143
gi|89199688|gb|dq398742.1|	(A)15	GGAGGAGTGTAAAGTGCCAAC	52.4	GGCTAATGTCGTGCGGAGG	63.2	58	417
gi|89199687|gb|dq398741.1|	(A)15	GGAGGAGTGTAAGTGCCAAC	55	GCCATTGTCGGAACGGAGG	63.2	58	417
gi|89199686|gb|dq398740.1|	(A)12	GGAGGAGTGTAAGTGCCAAC	55	GCCTTGTCGGAACGGTGGA	63.2	58	415
gi|89199685|gb|dq398739.1|	(A)12	GGAGGTGCTAAGTGCCAAC	57.9	GCCTTGTCGGAACGGTGG	66.7	58	415
gi|89199684|gb|dq398738.1|	(A)14	GGAGGTGCTAAGTGCCAAC	57.9	GCCATTGTCGGAACGGAGG	63.2	58	417
gi|89199683|gb|dq398737.1|	(A)14	GGAGGAGTGCTAAGTGCCAAC	57.1	GAGCCTATGTCGGAACGG	61.1	58	417
gi|89199682|gb|dq398736.1|	(A)10	GGGTCTCTAAGTGCCAAC	55.6	GCCAGTGCTCGGAACAGTG	63.2	56.55	411
gi|89199681|gb|dq398735.1|	(A)12	GGAGGTCTGTAAGTGCCAAC	55	GCGGATTGTGGAACGGCG	66.7	56.55	413
gi|89199680|gb|dq398734.1|	(A)12	GGAGGTGTAAGTGCCAACG	57.9	GGCTATGCTCAGTCAGGG	61.1	56.55	413
gi|89199679|gb|dq398733.1|	(A)12	GGAGGTGCTAAGTGCCAAC	57.9	GACATTGCTCGGAACAGGG	57.9	56.55	413
gi|89199678|gb|dq398732.1|	(A)15	GGAGGTGCTAAGTGCCAAC	57.9	GAGCCTTGCGAACGGTGG	66.7	58	418
gi|89199624|gb|dq398678.1|	(T)10	GTGTAGTCGGTTCCATCG	55.6	GTTGGTCGGAGGAGTCGC	66.7	58.02	462
gi|89199623|gb|dq398677.1|	(T)10	GTGGTAGTGGTATCATCG	50	GGATAAGTCGGAAGAGGC	55.6	58.02	462

Table 2 (contd...)

Accession number	Motif	Forward Primer		Reverse primer		Annealing temperature (C)	Product size (bp)
		Primer	GC %	Primer	GC %		
gi|89199624|gb|dq398678.1|	(A)21	CGTGTAGAGTGCCATCCG	61.1	GATGACTACGAGGATGGCG	57.9	58.05	462
gi|89199623|gb|dq398677.1|	(T)10	CGTGTAGTCGGTCCATCG	61.1	GTGATAGACAGAGGAGAGCG	55	58.05	462
gi|89199612|gb|dq398666.1|	(T)10	GGATGAGCAGAGGAGAGCC	63.2	GATAGAGTCAGAGGAGGGC	57.9	58.05	461
gi|45738090|gb|ay563392.1|	(TA)8	GGTGCGATAGACTCAACGG	57.9	GTATCGCTATCGCACAGTC	52.6	58.15	676
gi|45738088|gb|ay563390.1|	(TA)7(AT)7	GAGTCACAGTCGTCAGCG	61.1	GCGTGAGTATCGTAGCAGTC	55	58.15	639
gi|9994240|gb|af102469.2|	(A)14	GCTGCGTATGCGAGACAC	61.1	GCACGGGTATTTTCAGTCCTCGC	56.5	58.1	648
gi|9994232|gb|af102419.2|	(A)10	GATAGTGCTGACGAGTGCG	57.9	GCACGCAGTCAGTCTCGC	66.7	57.5	786
gi|9994224|gb|af102376.2|	(A)10	CACGCCAATCCTGACGCAC	63.2	GCACGGGTTTCAGTCCTCGC	65	58.85	779
gi|224985956|gb|fj014139.1|	(A)10(A)14	CTGGATGGAACCCTGAGTG	57.9	GCTTGACGCAGAACGGTG	61.1	55.45	356
gi|46403206|gb|ay596976.1|	(GAG)5	GGAGACGATTGGAGTTGGTG	55	CTCGCTTAGATACTCGCC	55.6	56	669
gi|7578882|gb|af240764.1|	(A)20	GGTGGAGGGCATAGAGGC	66.7	GGTGGAGGGCATAGAGGC	57.9	55.85	691
gi|6685069|gb|af205859.1|	(A)19	GAAGCCACCAGGAAGCAG	61.1	GTCAAATCACTTCCGCCAG	52.6	55.85	555
gi|6110321|gb|ah008318.1|	(A)12	GTATCGGCGTATGTGGGC	61.1	GCCAACCCATTCGTAAGTCC	55	57.8	550
gi|260079916|gb|gq245007.1|	(A)10	GGACACACAGCGAGCACG	66.7	GCGACGGTATTCACTCTCAC	55	57.95	320
gi|1644387|gb|u72654.1|	(A)23	GGCTGTTGGTAGATGGCTG	57.9	GGCGAACTCCTATGAACGG	57.9	57.8	690
gi|209483591|gb|fj232569.1|	(GC)7	CACTGCGATAGCGGACGAC	63.2	GCAGCATCTCTTCGGTGGGAC	61.9	61.35	602
gi|94317216|gb|dq449916.1|	(C)10	CGAATCCAGGCGAAGCAGACG	61.9	CGCTTACAGGTCGGCAGTCTC	61.9	62.75	608
gi|57634567|gb|ay858677.1|	(A)10	GGGAAACCACGGAGCGATG	63.2	GTGGCTTCGGACGCAACTG	63.2	60.1	307
gi|259435649|emb|hb880950.1|	(A)18	GAGAAGCCATAGGAGGTC	55.6	CGCAATACTCTCGTGACTCG	55	63.1	822
gi|205289952|gb|fj010824.1|	(TA)7	GCGGCAGAACTCAACGAC	61.1	GCGAGGCTATCCCACCAGTC	65	58.15	671
gi|205289938|gb|fj010810.1|	(TA)6	GTGGCAGGACTCAACGGC	66.7	GCACGAGCATCACGCCAGTC	65	58.15	669
gi|257693471|emb|hb769727.1|	(GAG)5	CTCGTCGTTGGCGGTGAATC	60	CAGCCATAACCTCACGGATAG	52.4	55.6	549
gi|241661579|dbj|ab453155.1|	(T)13(T)10	GGGAGTATTCTTATCGGAGCG	52.4	GCTGCTATTGATTGCCCGTC	55	54.95	312
gi|241661523|dbj|ab453127.1|	(T)10(T)10	GGGTTACTTATCGGGAATCG	50	CGATAGGCATTTTGGAGCGGC	57.1	58.55	331
gi|241661499|dbj|ab453115.1|	(T)11	GGGAGTTTATCGGGAATCG	52.6	CGTTAGGCGTTTTGGGCTG	57.9	58.3	331
gi|89511875|dbj|ab222605.1|	(T)10	CACGAGACTTGGGTTACGC	57.9	GAATCCCCCCAAACCGAGG	63.2	56.25	337
gi|89511875|dbj|ab222605.1|	(T)11	GAATAAGAGGACGCCACG	55.6	CGCAGAGCAAGCCCAATG	66.1	56.4	714
gi|62183686|gb|ay879942.1|	(A)13	GAATAAGAGGACGCCACG	55.6	GCTTGACGGCAGAACGGC	66.7	56.25	299
gi|166407456|gb|eu326062.1|	(T)12(GT)7	GAGCAGCAGACGAGTAGC	61.1	GACGACGCACATCTCCAC	61.1	57.25	320
gi|219929423|emb|fb899668.1|	(GAG)5	GGAGACGATGGAGTTGGTG	57.9	CATAGGTGACATACGCCG	55.6	56	669
gi|218478034|dbj|ab459662.1|	(T)12(GT)7	GCGACTATGGCTGCTGCTGC	65	CATAGGTGACATACGCCG	55.6	57.5	
gi|218478034|dbj|ab459662.1|	(T)17	GCACCGAGAAGCCAGCACCT	65	GAACATACTCTGCCCACCG	57.9	58.6	
gi|164454772|dbj|ab289445.1|	(ATG)5	CTTCTTCTACTCCGCAGC	55.6	GCAGAAGATGACTCCTCCAG	55	54.9	559
gi|164454770|dbj|ab289444.1|	(CTT)7	CCAGAAGTGAGGAAAGCG	55.6	GCAGTGACCAGAGACCCC	66.7	56.95	549
gi|193795409|gb|ef203258.1|	(TA)7 (T)15 (A)29	CGTGAGGATTGGCTGTCGGC	65	CTATGCGACCAGCGATTCAC	55	58.45	703
gi|193795407|gb|ef203257.1|	(G)14 (A)86	CAGAGCAAAGGAACCACCG	57.9	GGATGACGGCACCACCAAC	63.2	55.3	513
gi|113735151|dbj|ab271691.1|	(AT)6	GTAGCAGCAGTGTGGTCGGC	65	GATTTCAGACAACGACGGTG	50	55	677
gi|170673145|gb|eu541812.1|	(G)10	CGACCTTTGTAGCAGCCG	61.1	CGCTTTGTTGTGTGCCTTCG	55	55.15	499
gi|170673141|gb|eu541808.1|	(A)10	CACGACTCTCCCAGCACGC	68.4	GTCTCTGTCGTGTGCCTATCG	57.1	55.4	614
gi|170673139|gb|eu541806.1|	(T)10	CACGAATCATCCTCAGTCCTC	52.4	GTCTTTCGTGTGCCTTCG	55.6	55.4	611
gi|170672874|gb|eu528047.1|	(TAA)6 (T)10	GGGTAATCTGAGCCAAATCC	50	GCGAGGCTATCCCGACCAC	68.4	57.9	735
gi|4092183|gb|af102463.1|	(T)10 (A)10	CGGGTCGCAATCCTGAGCC	68.4	GCTTGACAGGCAGAACGGG	63.2	56.7	290

Table 2 (contd...)

Accession number	Motif	Forward Primer		Reverse primer		Annealing temperature (C)	Product size (bp)
		Primer	GC %	Primer	GC %		
gi|4092141|gb|af102421.1|	(A)11	GGGTCCGCATCCTGAGCC	72.2	GCTTGACGGCAGAACGGG	66.7	56.7	290
gi|4092124|gb|af102404.1|	(A)11	GCGGGTCGGATGTGAGCC	72.2	GCTTGACAGTGCGAACGGG	63.2	56.25	290
gi|133874211|dbj|ab190181.1|	(A)24	CGGGTCCTTGCGATGCCTGGG	71.4	CGTCCTCCTTCTCCACTGCC	65	58.3	592
gi|124388815|gb|ef069436.1|	(A)21	GGCTCAATGCGTCGGTAAC	57.9	GCCAGTCCAGTGAGTTCCG	63.2	57.7	137
gi83758482dbjAB19627.1	(TA)6	GGTTACGGTGAAGAGTGACAGG	54.5	GGATGGGAAGAGTGACAGG	57.9	58.25	374
gi|83758480|dbj|AB219625.1|	(TA)7	GGTTACGGAAGAGTGACAGG	55	CCATACCAAGGCTCAATCC	52.6	56.2	228
gi|156787487|gb|ef569229.2|	(AT)6 (A)20	CTTCTCCACGGTCGCCTTAC	60	GTGACTGAAGCATCCTACC	52.6	60.15	539
gi|156787485|gb|ef569228.2|	(A)18	GGATAGAGGCTGTGGGATGC	60	CACCAGTCTCAACACCTC	55.6	56.75	313
gi|146272406|dbj|ab281494.1|	(CAA)7	CGAGGATTCAAGTTCACGGC	55	GAGTTCAGGGACCGCATAGC	60	58.15	371
gi|147743640|gb|ef571643.1|	(CT)10	CCGTAGTGTTGGTCAGAAGCAGG	56.5	GCGACCTGTAGAACGGATGATG	54.5	57.85	350
gi|147743624|gb|ef571635.1|	(TC)6	GCAAGGCGAGCACCCAAG	66.7	GTTAGCCAGGATGCGAAGC	57.9	57.75	712
gi|147743612|gb|ef571629.1|	(TC)7	GCATTCGGTCAGCCAAGC	61.1	GGTGGTATTCATCTTCCGCCG	57.1	58.55	825
gi|147743609|gb|ef571627.1|	(CT)8	GCAACACAGCGGGACTAAC	57.9	GCACGACAGAACGAGCGG	66.7	60.25	617
gi|147743597|gb|ef571621.1|	(CT)11	CCTCGGACCACCAATCAGC	63.2	GTGTGAACGACTCCGCTTG	57.9	56.35	486
gi|147743595|gb|ef571620.1|	(CT)6	CAGTTTCGGTCAGCAAGC	55.6	GTGAGAACAACCCCCGCTG	63.2	57.35	440
gi|147743553|gb|ef571599.1|	(TC)7	GCAAAGAGGAGCACCAAG	55.6	GCAGGTATTCATCTCCGCCG	60	59.05	705
gi|44829182|gb|ay466118.1|	(A)28	GCCTACCCAAAACGCTGACC	60	CACCTGTCTTCTGTTAGCGG	55	59.2	645
gi|124295133|gb|ef203260.1|	(A)18	GTTGTGGAGGTGGCTTCG	61.1	CTGCGTAACACTCATCAAGCC	52.4	56.02	603
gi|118145133|dbj|dd357421.1|	(A)18	CCAAATGACGGACGCTACCC	60	CTATGCCACCAATGTCCC	55.6	57.35	791
gi|117935906|gb|ef062505.1|	(A)18	GGAGCCTATGCGAACGGG	66.7	GCGGTTCATTCTTACACGAGC	52.4	58.85	700
gi|95118583|gb|dq497593.1|	(A)11	CGTGTATCAAGGACCGCC	61.1	GGAGCCTATGCGAACGGG	66.7	56.35	345
gi|95118580|gb|dq497590.1|	(A)11	CTGTGTTCAAAGGACCCGTGC	57.1	GAGCCTATGTCGGAACGG	61.1	56.35	345
gi|13018492|dbj|e31220.1|	(A)18	CTCTGTGCTTTGTGATGCTG	50	GTGTATTTGTAGCGGCAGC	52.6	54.8	650
gi|92242664|dbj|bd340504.1|	(G)14(A)86	GGCTGGTGACTACTGCTG	61.1	CCGCACTCCAGCACGAAGC	68.4	58.95	561
gi|92128002|dbj|bd289870.1|	(A)15	CCTTGGTTGTGCTGTCCTGGTG	59.1	GAAGGCAACAGAGGTGACGC	60	58.6	447
gi|90959871|dbj|ab027191.1|	(T)14	GAGGATACGACCACGGACG	63.2	CAGAGCGTAGAAGCCATCAG	55	55.7	704
gi|90959867|dbj|ab027189.1|	(A)23	GATACCCCACAGGAGAACC	57.9	GACAACTCCCCAGCAAGAC	57.9	55.45	680
gi|90959865|dbj|ab027188.1|	(A)27	GCGAACTGCTACTCAAACCACC	54.5	CATTGAGCGACATCCCTGC	57.9	55.35	693
gi|90959863|dbj|ab027187.1|	(A)18	CGACATCAACCCCTACGCTG	60	GCCATAAGGGACGCCATTG	57.9	57.1	730
gi|6681691|dbj|ab017370.1|	(TA)7(T)15	CCTGTAACTCCCACCTCAACC	57.1	GCTGTCAATGTAGTCCTGCTTCG	52.2	57.9	679
gi|6681687|dbj|ab017368.1|	(TA)8	GGTAGTAAGTCCAGGTGCTCG	57.1	CGCTTCTTCACTCCCTTTGC	55	56.95	590
gi|89276224|gb|dq402068.1|	(T)14	CGCACCGAATAAAGCCACAG	55	GGTGAGAAGGAGACGGTGG	63.2	58.15	320
gi|86451166|gb|dq358898.1|	(T)10	GCCGTCAAATCTCTTCTCCTCG	54.5	CGGGTTCTTGTTGGCTGGCTATG	56.5	60.1	658
gi|76443559|dbj|ab011014.1|	(A)18	GTTGTGGAGGTGGCTTCGG	63.2	GGCTGAATGGTTGAGAGACG	55	56.6	446
gi|75812155|dbj|d38043.1|	(T)12	GCGAGGCATAAGACTGGCTG	60	CAACCGCAACAGGACACGC	63.2	57.2	681
gi|74267409|dbj|ab208689.1|	(A)10	GAATGGCAAGTGGTCGCTGG	60	GTATGGCTCGGCAAAGGTTC	55	54.8	460
gi|18146806|dbj|ab028666.1|	(G)13	GCAAGCAAGGGAATCAGG	55.6	CCGCACTCCAGCACGAAGC	68.4	58.6	629
gi|6687482|emb|aj236195.1|	(A)10	GTGACTTCGTGAGGACAGC	57.9	GTCGCAATGTCGTCGCAGG	63.2	56	453
gi|62433122|dbj|d38168.1|	(CT)8	CAGTCTCCTCCGTCACCGAAG	61.9	GAATGGAATCACGGGAG	52.9	56.75	450
gi|62086548|dbj|ab193314.1|	(A)28	GAATCATCGCCATCTCCACC	55	GTCCATAACCCTTTCAGCC	52.6	55.5	362
gi|62086546|dbj|ab193313.1|	(A)12(A)12	GGTGTTAGCGGGCATTCG	61.1	CAGACGCTCCACCTTCCC	66.7	57.25	524
gi|62086540|dbj|ab193310.1|	(A)19	GAATGGCAAGTGGTCGCTGG	60	GTATGGCTCGGCAAAGGTTC	55	54.8	620
gi|38568130|emb|aj489906.1|	(C)10	GATGATTGCCGCCTGCTGG	63.2	GGACTCGCATTTAGCCAGC	57.9	59.8	349

Table 2 (contd...)

Accession number	Motif	Forward Primer		Reverse primer		Annealing temperature (C)	Product size (bp)
		Primer	GC %	Primer	GC %		
gi|4455871|emb|aj010509.1|	(T)12	TGGGTAAAAGATGCTCCGTC	50	CCATAGGTAAGTCCGAAAGTCC	50	55.15	317
gi|37954874|gb|ay251732.1|	(A)19	GGTGCCAATCCTGAGCCG	66.7	GCTTGACGGCAGAACGGG	66.7	56.4	307
gi|37954873|gb|ay251731.1|	(A)16	GGATTGAGCCTGGTATGG	55.6	GCCTTGACAGCAGAACGGG	63.2	56.5	375
gi|37991673|dbj|ab124878.1|	(A)16	GCAGTAAGGGAGCGTGAC	61.1	GTCGTCAAGTCAGCCAAC	55.6	54.6	460
gi|22773822|dbj|ab080739.1|	(A)13	CATAGGAAGGCAAGGAAGC	52.6	GATTCAGGTAGCAACGGAGTGG	54.5	58.9	528
gi|33945370|emb|aj490190.1|	(A)10	GGTGGCAGGACTCAACGG	66.7	GCGATTGACAGCAGAACGGG	60	56.95	204
gi|15149940|emb|aj315324.1|	(A)11	GTCTGCCTGCCGATTTGGG	63.2	CTACACAGGTTCGGAGGG	61.1	62.4	75
gi|27530874|dbj|ab076697.1|	(A)18	GATTCAGCAGCCGAGGAG	61.1	GTTATGCCTCCGCTCAGTG	57.9	55.35	458
gi|22796301|emb|aj430909.1|	(AT)7	GATGGCAGTCCTCCGCTTC	63.2	CCGTTGATGTCTCTGCTACC	55	55.95	597
gi|18146804|dbj|ab028665.1|	(G)14(A)86	CAGTTCCATCAAGCACCAGGCG	59.1	CTTCCGTGCGATGTCAGG	61.1	56.7	622
gi|1785485|dbj|d14589.1|	(A)15	GGCTGTTGGAAATGGCGG	61.1	GAGCGTTTATTCTGGGGC	55.6	56	672
gi|3808127|emb|aj011983.1|	(A)12	CCGTAGGGTTGGGTCTTC	61.1	GCCAACCATTCGTAAGTCC	52.6	56.05	527
gi|10189942|emb|ax028840.1|	(A)17	GGTCATTACTCGGGGTGTG	57.9	GCACTCTCACATCACATTGGGC	54.5	56.6	797
gi|7415596|dbj|ab026494.1|	(TA)6(A)21	CTTGAGAAATGCCGTGTTGC	50	CTACAAATGCGGCTCCTCAC	55	54.8	410
gi|221150824|gb|gh694656.1|	(C)12	GGAGGAGTGAATCGGAACCC	60	GTGAGGTGGAGGGACTGG	66.7	58.55	181
gi|62086548|dbj|ab193314.1|	(A)28	GAATCATCGCCATCTCCACC	55	GTCCATAACCCTTTCAGCC	52.6	55.5	362
gi|62086546|dbj|ab193313.1|	(A)12(A)12	GGTGTTAGCGGGCATTCG	61.1	CAGACGCTCCACCTTCCC	66.7	57.25	424
gi|62086540|dbj|ab193310.1|	(A)19	GAATGGCAAGTGGTCGCTGG	60	GTATGGCTCGGCAAAGGTTC	55	54.8	620
gi|38568130|emb|aj489906.1|	(C)10	GATGATTGCCGCCTGCTGG	63.2	GGACTCGCATTTAGCCAGC	57.9	59.8	349
gi|33945346|emb|aj489882.1|	(C)10	CACAAACGGCGACGAGAAG	57.9	CAGCACACGAGTTGAGGC	61.1	61.25	445
gi|4455871|emb|aj010509.1|	(T)12	TGGGTAAAAGATGCTCCGTC	50	CCATAGGTAAGTCCGAAAGTCC	50	55.15	317
gi|37954874|gb|ay251732.1|	(A)19	GGTGCCAATCCTGAGCCG	66.7	GCTTGACGGCAGAACGGG	66.7	56.4	307
gi|37954873|gb|ay251731.1|	(A)16	GGATTGAGCCTGGTATGG	55.6	GCCTTGACAGCAGAACGGG	63.2	56.5	375
gi|37991673|dbj|ab124878.1|	(A)16	GCAGTAAGGGAGCGTGAC	61.1	GTCGTCAAGTCAGCCAAC	55.6	54.6	460
gi|22773822|dbj|ab080739.1|	(A)13	CATAGGAAGGCAAGGAAGC	52.6	GATTCAGGTAGCAACGGAGTGG	54.5	58.9	728
gi|33945370|emb|aj490190.1|	(A)10	GGTGGCAGGACTCAACGG	66.7	GCGATTGACAGCAGAACGGG	60	56.95	204
gi|15149940|emb|aj315324.1|	(A)11	GTCTGCCTGCCGATTTGGG	63.2	CTACACAGGTTCGGAGGG	61.1	62.4	75
gi|27530874|dbj|ab076697.1|	(A)18	GATTCAGCAGCCGAGGAG	61.1	GTTATGCCTCCGCTCAGTG	57.9	55.35	458
gi|10189940|emb|ax028838.1|	(A)17	CGGGCTAAGAGACACGGC	66.7	GGTGTGGCTCGTTGAATGCG	60	59.3	285
gi|22796301|emb|aj430909.1|	(AT)7	GATGGCAGTCCTCCGCTTC	63.2	CCGTTGATGTCTCTGCTACC	55	55.95	597
gi|18146804|dbj|ab028665.1|	(G)14(A)86	CAGTTCCATCAAGCACCAGGCG	59.1	CTTCCGTGCGATGTCAGG	61.1	56.7	522
gi|1785485|dbj|d14589.1|	(A)15	GGCTGTTGGAAATGGCGG	61.1	GAGCGTTTATTCTGGGGC	55.6	56	672
gi|3808127|emb|aj011983.1|	(A)12	CCGTAGGGTTGGGTCTTC	61.1	GCCAACCATTCGTAAGTCC	52.6	56.05	427
gi|10189942|emb|ax028840.1|	(A)17	GGTCATTACTCGGGGTGTG	57.9	GCACTCTCACATCACATTGGGC	54.5	56.6	797
gi|7415596|dbj|ab026494.1|	(TA)6 (A)21	CTTGAGAAATGCCGTGTTGC	50	CTACAAATGCGGCTCCTCAC	55	54.8	410
gi|221150824|gb|gh694656.1|	(C)12	GGAGGAGTGAATCGGAACCC	60	GTGAGGTGGAGGGACTGG	66.7	58.55	181

clustering patterns, test for Hardy-Weinberg equilibrium and multilocus equilibrium, and to test polymorphic loci for the evidence of selective neutrality. This can be useful to plant breeders, germplasm managers, or others who are interested in population genetic properties of materials that they are working with. The three most common types of markers used today are RFLP, RAPD, and microsatellites. A wide variety of methods for the construction of libraries enriched for microsatelite sequences have been reported, the most popular among those being the ones based on vectorette PCR using anchored primers. But this method is highly time-consuming and expensive, and the alternative is to use bioinformatics, that is, computational tools to screen the public database and find SSR. EST-derived molecular markers, especially SSR and SNP, are highly useful in developing linkage maps and markers assisted breeding programs. These markers are also transferable to related genera.

Molecular marker techniques are advantageous as they directly reflect variations in the DNA sequences and therefore of independence of environment. Among many molecular marker techniques currently available, microsatellites and SSRs [35] provide an improved technology in assessing genetic diversity and genetic relationships in plants as they are highly polymorphic, codominants, very informative, and PCR based. EST-SSRs offer the following advantages over other genome DNA-based markers: (1) they should detect variation in the expressed portion of the genome so that gene tagging should give “perfect” marker–trait associations; (2) they can be developed at no cost from the EST databases; and (3) once developed, these markers, unlike genomic SSRs, may be used across a number of related species. With the growth of sequence databases, several authors have reported an abundance of SSRs in different genomes. The Distribution of SSRs in the rice genome has also been studied on the basis of the two whole genome draft sequences released, respectively, by Syngenta and by the Beijing Genome Institute (BGI). In the draft sequence released by Syngenta, for instance, 48,351 SSRs (including di-, tri-, and tetranucleotide repeats) were available, giving a density of 8 kb per SSR in the whole genome; SSRs represented by di-, tri-, and tetranucleotide repeats accounted respectively for 24%, 59%, and 17% of the total SSRs.

SSRs are very polymorphic due to the high mutation rate affecting the number of repeat units. Such length-polymorphisms can be easily detected on high-resolution gels (e.g., sequencing gels), by running PCR-amplified fragments obtained using a unique pair of primers flanking the repeat.[36] Chung and Staub[37] developed a set of consensus chloroplast primer pairs for ccSSRs from *N. tabacum* chloroplast sequences. All primer pairs produced amplicons after PCR employing chloroplast DNA from members of the *Cucurbitaceae* (six species) and *Solanaceae* (four species). Sixteen, 22, and 19 of the initial 23 primer pairs were successively amplified by PCR using template DNA from species of the *Apiaceae* (two species), *Brassicaceae* (one species), and *Fabaceae* (two species), respectively. Twenty of the 23 primer pairs were also functional in three monocot species of the *Liliaceae* (onion and garlic), and the *Poaceae* (oat). ccSSR primers were strategically "recombined" and referred to correctly as recombined consensus chloroplast primers (RCCP) for PCR analysis of cucumber DNA such that the primers designed for the SSR-containing genus of Gentiana family would be utilized for the production of amplicons from different members of family.

Kijas *et al.*[38] tested two primer sets in 10 different *Citrus* species and two related genera and found conservation of the sequences. Cross-species amplification has also been reported between cultivated rice and related wild species[39] and between *Vitis* species.[40] Provan *et al.*[41] could show successful amplification of two tomato SSR primer pairs tested on potato cultivars. Weising *et al*,.[42] reported conservation of SSR flanking sites in different species of kiwifruit (*Actinidia chinensis*). Usually, a low percentage of markers also amplified fragments from species belonging to other genera from the same family. Within the *Poaceae* family, primers worked even across different genera,[43] but only 50% of microsatellite loci identified in wheat were also polymorphic in rye and barley cultivars. Whitton *et al.*[44] tested 13 SSR loci in 25 representatives of the *Asteraceae*, where it was demonstrated that the regions flanking in the repeats are not highly conserved, neither in the nucleotide sequence nor in the relative position.

Indeed, in general, transferability of polymorphic markers in plants is likely to be successful mainly within genera (success rate close to 60% in eudicots and close to 40% in the reviewed monocots) rather than between genera (transfer rates are approximately 10% for eudicots) within the same family.[45] This transferability of polymorphic markers nature in plant generally enhances the utilization of the primers in random way. Comparative genome analysis facilitates high-throughput comparative mapping with the assistance of cross-species markers, and further facilitates gene cloning by identifying cross-reference genes. Seventeen SSR primer sets developed for *Quercus petraea* were tested on eight different members of the *Fagaceae* family.[46] In total 66% resulted in interpretable amplification products and most of them were really homologous to the originally cloned SSR fragment from *Q. petraea*. The primers could be designed successfully for a very large number (169, 31%) of SSRs [Table 2]. However, it was not possible to design the primers for remaining SSRs (376, 69%) because the sequence flanking at both ends of the SSRs was inadequate in size to design the primers. The large number of primer pairs for the SSRs that have been designed during the present study may be utilized for a variety of purposes, for example, gene tagging, genetic mapping, population studies, etc. Due to a high level of potential for length polymorphisms, SSRs have become a valuable source of genetic markers and have been broadly applied to various areas of genetic research including studies of genome variation, establishment of genetic maps, integration of physical and genetic maps, determination of evolutionary relationships, and comparative genome analyses.

CONCLUSIONS

Nucleotide sequences of Gentiana family were systematically searched for SSRs using the "ssr_finder.pl" perl program for the development of SSR markers. This is a valuable approach for both costs and time, given a sufficient amount of available Gentiana family sequences. The use of SSRs in genetic diversity studies is a novel tool that reveals variation in genomes.

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