Microsatellite analysis in the genome of *Acanthaceae*: An *in silico* approach

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ABSTRACT

Background: *Acanthaceae* is one of the advanced and specialized families with conventionally used medicinal plants. Simple sequence repeats (SSRs) play a major role as molecular markers for genome analysis and plant breeding. The microsatellites existing in the complete genome sequences would help to attain a direct role in the genome organization, recombination, gene regulation, quantitative genetic variation, and evolution of genes. **Objective:** The current study reports the frequency of microsatellites and appropriate markers for the *Acanthaceae* family genome sequences. **Materials and Methods:** The whole nucleotide sequences of *Acanthaceae* species were obtained from National Center for Biotechnology Information database and screened for the presence of SSRs. SSR Locator tool was used to predict the microsatellites and inbuilt Primer3 module was used for primer designing. **Results:** Totally 110 repeats from 108 sequences of *Acanthaceae* family plant genomes were identified, and the occurrence of dinucleotide repeats was found to be abundant in the genome sequences. The essential amino acid isoleucine was found rich in all the sequences. We also designed the SSR-based primers/markers for 59 sequences of this family that contains microsatellite repeats in their genome. **Conclusion:** The identified microsatellites and primers might be useful for breeding and genetic studies of plants that belong to *Acanthaceae* family in the future.

Key words: *Acanthaceae,* microsatellites, molecular markers

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Acanthaceae is one of the major groups in angiosperms and the plants, which belong to this family are mostly tropical herbs, shrubs, and rarely trees.[1] *Acanthaceae* family has about 202 genera and 3520 species, and most of this family members are well-known medicinal plants (e.g. *Acanthus ilicifolius*, *Andrographis paniculata*, *Asteracantha longifolia*, *Hemigraphis hirta*, *Justicia adhatoda*, *Nelsonia canescens*, etc.) used in traditional medicine as they possess biologically active phytochemicals.[1-3] The medicinal plants of this family are enriched with secondary metabolites such as alkaloids, flavanoids, glycosides, steroids, saponins, phenols, and tannins. The phytocompounds obtained from these plants are used to treat diarrhea, snake bite, fever, inflammation, hypertension, diabetes, malaria, jaundice, and rheumatism.[1,3,4]

Simple sequence repeats (SSRs) or microsatellites are the short DNA sequences with 1-6 base pairs of length.[5]

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Several studies suggest that the abundance of SSRs were present in noncoding regions of the genome sequences.[6] These repeats have a wide application in the field of plant genetics and breeding as they have multi-allelic, reproducible and co-dominant inheritance properties.[7] The microsatellites were identified in the genomes of many medicinal plants such as *Humulus lupulus* (hop),[8] *Ocimum basilicum* (basil)[9] and also for some economically important plants including *Oryza sativa* (rice),[10] *Glycine max* (soybean),[11] *Hordeum vulgare* L. (barley), *Triticum aestivum* (wheat), *Solanum lycopersicum* (tomato), *Vitis vinifera* (grape), and *Helianthus annuus* (sunflower).[9] The SSRs also provide basis for the development of SSR-based markers that have a wide range of application in genetic research including studies of genetic variation, linkage mapping, gene tagging, and evolution.[7]

The objective of this study includes identification of microsatellites and its associated primers in the genome of *Acanthaceae* family. In the current study, the different types of SSRs and its distribution from 108 sequences and 59 associated primers were identified. We also reported that the amino acid isoleucine was enriched in all the above SSRs in the genome sequences of *Acanthaceae* family.

MATERIALS AND METHODS

Nucleotide sequence retrieval

Totally 4077 nucleotide sequences were retrieved from National Center for Biotechnology Information for *Acanthaceae* family plants.[12]

Identification of simple sequence repeat motifs

In order to identify the microsatellites from the genome of *Acanthaceae* family, the SSR Locator computing tool was used.[13] It is integrated with the functions such as primer design and PCR simulation.[14] The sequences were searched for mono-, di-, tri-, tetra-, penta- and hexa-types of SSR motifs with number of repeats 20, 10, 7, 5, 4, and 4, respectively. The enriched amino acids in the predicted SSRs were also identified using the above mentioned SSR Locator tool.

Simple sequence repeat-based primer designing

The *in silico* based primers for each microsatellite containing nucleotide sequences were designed using Primer3, the interface module in the SSR Locator.[14] The default parameters such as length of the primer (15-25 base pairs), melting temperature (45-55°C) and GC content (45%) were specified for primer designing.

RESULTS

Simple sequence repeat identification and distribution in the genome

The SSR motifs were identified from 4077 nucleotide sequences of the *Acanthaceae* family plants with 31,41,420 base pairs. Totally we found 110 SSR motifs from 108 nucleotide sequences using SSR Locator tool. The microsatellites such as mononucleotide (32%), dinucleotide (50%) and tetranucleotide (11%) were found abundant in number in *Acanthaceae* family plants. The trimer, pentamer and hexamer motifs occurred comparatively less (about 2%) in the nucleotides. A summary of the predicted SSRs in the sequences was shown in Table 1. The distribution of SSR motifs in the *Acanthaceae* genome was shown in Figure 1.

The mononucleotides, including A and T were found abundant in the *Acanthaceae* family genomes. We also found eight different dinucleotide repeats (TG, AC, CA, AG, GT, TC, GA, and CT), which were found in about 50% of this genome. TCA, ATC, CAT were identified as trinucleotide repeats and the ATC repeat showed a higher frequency. There were nine tetranucleotide repeats such as AGAC, TATG, ATAC, ACAT, GTAT, TAGA, TATT, CTAT and ATAG with the frequencies of 5, 12, 9, 11, 8, 5, 5, 7, and 5, respectively. The occurrences of pentanucleotide and hexanucleotide are comparatively less in number. TTGAT, a pentanucleotide and two hexanucleotides TTTCTT and TATATC were found in the *Acanthaceae* family genomes with frequencies of 8, 4, and 5, respectively. The distribution of above mentioned repeats (from mono- to tetra-) were shown in Figure 2.

Frequency of amino acids in the microsatellites

The combination of three nucleotides/triplet codon generally codes for a specific amino acid type. In this study, we found that the trinucleotide repeat ATC was enriched in this genome and it predominantly codes for the essential amino acid isoleucine. The other SSR motifs encode the amino acids including histidine, serine, tyrosine, phenylalanine, leucine, arginine, and lysine that occupied the next levels of this genome. The types of SSRs along with codon and its specific amino acids were shown in Table 2.

Simple sequence repeat-based primers/markers

The designing of SSR-based primers/markers through experiments are costly and time consuming as well. Hence, using *in silico* primer designing methods, 59 out of 108 nucleotide sequences exhibited the primers. The designed

Table 1: Summary of the screened microsatellites in the genome of *Acanthaceae*

Parameters	Values
Total number of sequences examined	4077
Total size of examined sequences (bp)	31,41,420
Total number of identified SSRs	110
Number of SSR containing sequences	108
Number of sequences containing more than 1 SSR	2
Mono-repeats	36
Di-repeats	55
Tri-repeats	3
Tetra-repeats	12
Penta-repeats	2
Hexa-repeats	2

SSRs: Simple sequence repeats

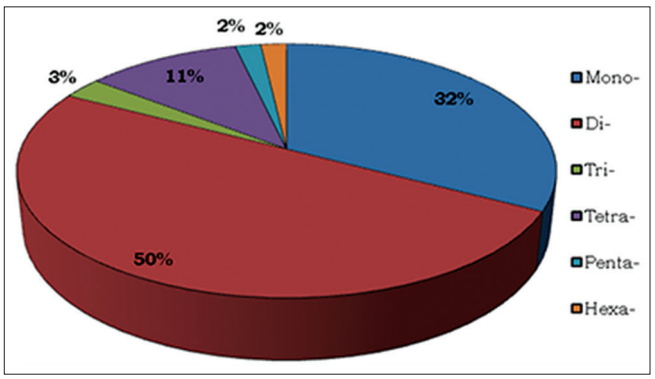

Figure 1: Frequency of simple sequence repeat motifs in the nucleotide sequences of *Acanthaceae* family plant genomes

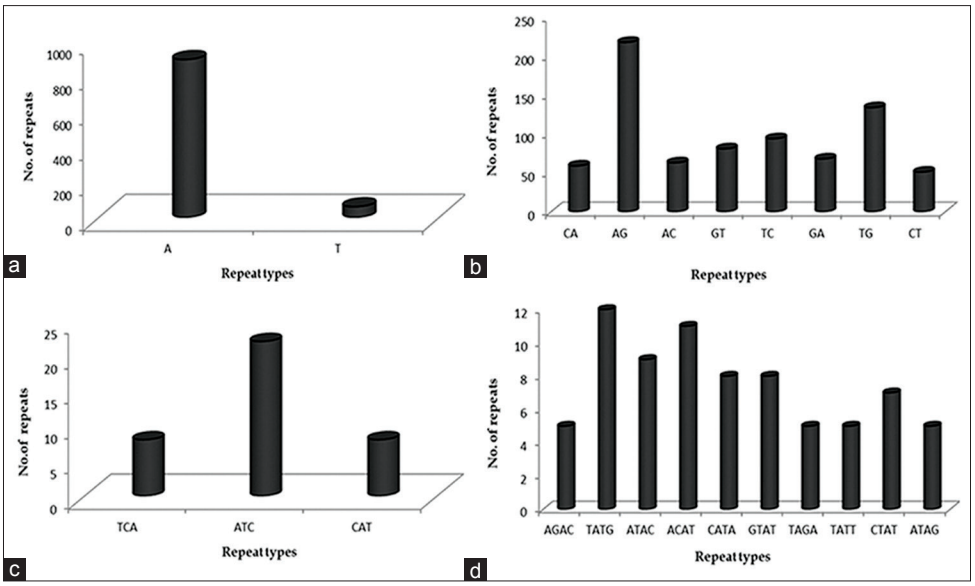

Figure 2: Frequency distribution of (a) mononucleotide, (b) dinucleotide, (c) trinucleotide, and (d) tetranucleotide repeats in the genome of *Acanthaceae*

Table 2: Microsatellites and its corresponding amino acids in the genome sequences of *Acanthaceae* family plants

Microsatellite	Number of bases	Number of repeats	Codon	Amino acid
ATC	3	22	AUC	Ile
CAT	3	8	CAU	His
TCA	3	8	UCA	Ser
TATATC	6	5	UAU/AUC	Tyr/Ile
TTTCTT	6	4	UUU/CUU	Phe/Leu
ATATATAGA	9	3	AUA/ UAU/AGA	Ile/Tyr/Arg
TTCTAAAAA	9	3	UUC/ UAA/AAA	Phe/Stop codon/Lys

forward and reverse primers of this genome also have adequate annealing temperature and GC content as well. The nucleotide accession numbers, forward and reverse primer sequences, melting temperature and product size of the designed primers were given in Table 3.

DISCUSSION

The genome sequences of *Acanthaceae* family plants were retrieved and examined to know the occurrence of microsatellite types, characteristics and distribution. The length of microsatellites 1-6 base pairs were taken into consideration. Totally, we screened 110 SSRs from 108 out of 4077 nucleotide sequences. The occurrence of dinucleotide repeats was comparatively higher than all other repeat types including mono-, tri-, tetra-, penta-, and hexa-repeats and the same repeats pattern was reported in *Arabidopsis thaliana*.[15,16] Among mononucleotides, A/T repeat was found to be abundant than C/T repeat in which repeat A (94%) is higher than T repeat. The dinucleotide repeat AG showed a higher frequency (28%) among eight other dinucleotide repeats such as TG (17%), TC (12%), GT (11%), GA (9%), AC (8%), CA (8%), and CT (7%) in the *Acanthaceae* family plants. The studies on medicinal plant *O. basilicum* and other plants, including *Arabidopsis thaliana*, *T. aestivum*, *H. vulgare* L., *O. sativa*, *Zea mays*, and *Prunus dulcis* also proposed that the frequency of AG repeats was higher than other dinucleotide repeats.[9] The trinucleotide repeat ATC (52%) was observed high in number than other trinucleotide repeats TCA (14%) and CAT (14%). The same trinucleotide repeat pattern was reported in *Gossypium* (cotton) (*Gossypium arboretum*, *Gossypium raimondii*, and *Gossypium hirsutum*) and *Arabidopsis thaliana* genomes.[16,17] The AT rich repeats were abundantly found in tetranucleotides of this family genome. The penta- and hexa-repeats were found in less frequency compared with other repeat types. We also identified that the repeat ATC, which codes for the essential amino acid isoleucine were found to be enriched in trinucleotide to hexanucleotide repeats and the amino acids serine and histidine were seen in less frequency in this genome. It was reported that the same amino acid distribution pattern was seen in the genome of *H. lupulus*.[8] The amino acid isoleucine plays a major role in maintaining blood sugar level. Therefore the *Acanthaceae* species such as *Adhatoda vasica*, *A. paniculata*, *A. longifolia*, *Barleria cristata*, *Barleria noctiflora*, *Barleria prionotis*, *Dipteracanthus prostrates*, *Jacobinia suberecta*, and *Strobilanthes crispus* have been used to treat diabetes in the traditional system of medicine as they contain enriched isoleucine in their genomes.[18] Moreover, the plants such as *Hygrophila auriculata*, *N. canescens*, and *Peristrophe bicalyculata* were also used to treat various infectious diseases such as diarrheal diseases, cholera,

Table 3: SSR-based markers of *Acanthaceae* family genome sequences

Contigs	Forward primer	Temperature forward (°C)	Reverse primer	Temperature reverse (°C)	Product size (bp)
gi|441482147|gb|KC118349.1	TTTGCTTATCTTCCATAAAG	50.105	TGAGATAATCTTTGGAATTG	50.305	275
gi|147883767|gb|EF214648.1	CTTCTTCCCTTTTTCTAAGT	49.881	CTTGAATTGCAAACAAAA	50.503	174
gi|167595818|gb|EU431055.1	AATTCCTTATTTGGCTATCT	50.127	GTCTATCCTCTCTGGTCTTT	49.896	138
gi|167595792|gb|EU431029.1	CAAAATCAACACAAAGAGAT	50.166	GTAAGGAGTCTGTCTTCCTC	50.331	225
gi|167595823|gb|EU431060.1	AATTCCTTATTTGGCTATCT	50.127	CCTCTCTGGTCTTTCTCTAT	50.124	130
gi|158668364|gb|EU081072.1	AAATGCATTGTGTAAGAATC	50.231	ATTCTCTATTCAAGCAACAA	50.123	117
gi|307950703|gb|HM470027.1	ATAAACAAATGCACTTCAAT	49.944	CATAAGTTGCCTGTAAAAAT	49.886	263
gi|307950699|gb|HM470023.1	ACTAGAGCATAGCTTTTGTG	50.024	CTTTAAATTCATTGGTGATG	50.759	148
gi|307950693|gb|HM470017.1	CACAAAATGCACATAAATAA	49.829	ACTGAATGTCATAATGTGTG	48.677	229
gi|307950691|gb|HM470015.1	GTTCACTGCTTACTGTCATT	50.134	AAGGCCTTTCTATCATAAGT	50.082	271
gi|307950689|gb|HM470013.1	ATAAACAAATGCACTTCAAT	49.944	CATAAGTTGCCTGTAAAAAT	49.886	263
gi|307950683|gb|HM470007.1	GAACAGTTGCTCTATACTGG	49.929	AGGAGAAATAAAAGTTGGTT	49.981	259
gi|307950700|gb|HM470024.1	ACTTCTTGATTCTAATGGGT	50.407	AACCAAGAACAAAACAGTAA	50.008	105
gi|307950696|gb|HM470020.1	ATATTCACAAGGAACAGTTG	50.022	AGGAGAAATAAAAGTTGGTT	49.981	270
gi|307950690|gb|HM470014.1	TGAGAAGGATGTTAAAAGAA	50.106	TAACACACGAAAGGTTAAAT	50.106	240
gi|307950680|gb|HM470004.1	GTTCACTGCTTACTGTCATT	50.134	AAGGCCTTTCTATCATAAGT	50.082	271
gi|187950111|gb|EU528996.1	AATTCCTTATTTGGCTATCT	50.127	CCTCTCTGGTCTTCTCTATT	50.124	159
gi|87133115|gb|DQ372453.1	TTGGAAATTCTTTTTGTTAC	49.791	CTCTTACTGCTATTTTTCCA	50.058	240
gi|66815240|gb|DQ059262.1	TTGAATAAAAAGCAGTCAAT	50.298	TTGTGTTGATATTGTTCAAA	49.814	266
gi|476002153|gb|JX461339.1	GTATCCTATTCGATTGTCAG	49.654	TACTAAAGGGGCATATACAG	49.839	272
gi|401716762|gb|JX445148.1	GTATCCTATTCGATTGTCAG	49.654	TACTAAAGGGGCATATACAG	49.839	272
gi|343530772|gb|HQ172893.1	GATACCTTTGGTTACACAGA	50.022	CACTCATATCGGACAACTAT	49.937	209
gi|343530770|gb|HQ172891.1	TCTCAACTGATTACCTCAAC	49.977	GGATAAACCACTTTGTATCA	50.073	206
gi|343530771|gb|HQ172892.1	CTCACAGTCCAAAATATAGC	49.982	ATATTCACACAGGTTGTTGT	50.082	266
gi|343530769|gb|HQ172890.1	CATGAATTGGAACAATAACT	50.073	CCAATCAAGATGCAAAT	49.603	121
gi|78499527|gb|DQ240231.1	AATGCACAATAAACAAATTC	50.402	AATTTCTGCATACTTCTTGA	50.123	257
gi|78499525|gb|DQ240229.1	CCAATTTCATGTCATAGTTT	50.073	TTGAGTGATTTTCATTTCTT	49.882	173
gi|78499523|gb|DQ240227.1	GCAATGAAATTACAAAAGAG	50.298	ATTATTTGTTTGGAAGTGTG	50.205	137
gi|78499521|gb|DQ240225.1	TCATGTCATAGTTTTTCTCC	50.03	TTGAGCTTAAATATCATTCC	49.764	202
gi|78499519|gb|DQ240223.1	TAATTCTGAAGAAGAGTGGA	49.92	TATAGTCAATTCGGCTACTC	49.757	199
gi|78499517|gb|DQ240221.1	TTCTTAGCGTGGTAAGTAAG	50.232	CTTGACAGTGTTATCCATTC	50.326	241
gi|54292642|gb|AY741808.1	ATTTTCACCTATATGAATGG	49.254	ACGTGCATATATTTTTGAGT	49.993	168
gi|54292640|gb|AY741806.1	ATTATGACTAAAATGCTCCA	50.033	AATTAAATAAAGGGAGAAGC	49.993	218
gi|54292638|gb|AY741804.1	AATCAAGTTGCTCAAGTAAA	50.254	TGTTAACAGGTTTTTCAAAT	50.058	222
gi|54292636|gb|AY741802.1	AACGCAGATAAATCCCT	50.241	TGAAAAGAGAGAGATGTTTG	50.359	151
gi|54292634|gb|AY741800.1	TTTTCTCAGAGATGCTAGAC	49.592	CATATTAGGACTTGGAAACA	50.295	278
gi|33439455|gb|AY281862.1	AACAATGAAAGGATTTTGTA	49.928	TATGACAGAAGGTTTGTCTC	49.977	275
gi|33439453|gb|AY281860.1	ATTATCCTAACCAATCCTTC	50.043	GGACTAACAAACATGAGAAA	50.166	238
gi|33439451|gb|AY281858.1	GCTGAATATACCTTTCTGTG	49.982	GCTCATTATTGAAACTATGG	50.033	211
gi|30961874|gb|AY283927.1	TCCTTCTGTTCTTTAACTGT	49.06	CGACTTCTAGCAACTAACAC	50.257	211
gi|78499528|gb|DQ240232.1	AAGATTACCCCATTTATTTT	49.948	AACTTTCTATAAAGGGGAAG	49.802	194
gi|78499526|gb|DQ240230.1	TGTATGAAATCTAGGTGGAG	50.116	AAAGGACATTATTTATGGTG	49.389	251
gi|78499524|gb|DQ240228.1	CACCATCAAAGAATTACACT	50.022	GTTTAGCCTGATAAGTTGAG	49.375	183
gi|78499522|gb|DQ240226.1	TATGAATTACGGCAAGTATT	50.254	TACAATAGATCGATCACACA	49.514	152
gi|78499520|gb|DQ240224.1	TATTTGGTTGTATTTGGTTT	49.972	GATTGACCATTAGACTTGAA	50.03	279
gi|78499518|gb|DQ240222.1	TTGCTAGAATAGCAGAATTT	49.806	ACACAAATAACAATTCCAAC	49.982	233
gi|78499516|gb|DQ240220.1	ACTTGAGATTCACATAGCAC	49.994	TGTATTCACCTATTTGATCC	49.94	257
gi|54292635|gb|AY741801.1	TGGCATTTCTGTATTTATCT	50.033	AGCAACTACCAAGTGAGTAA	50.065	226
gi|54292633|gb|AY741799.1	CCTAATTTATTTGATGGAAA	49.796	CCTCAAGTGCATCTTATTAC	49.982	110
gi|33439454|gb|AY281861.1	CAGATGAAACTCAAGAGAAG	49.863	TACTGATTGAGACTGGTAGG	50.064	227
gi|33439452|gb|AY281859.1	TATTTTTCTTGTCTTTTTGG	50.004	GAGATATTTCAATCCATCCT	50.616	204
gi|33439450|gb|AY281857.1	GAAGGCTTTAAAATAGGATT	49.993	AAAATTTCAAAATCACAAGA	50.066	152
gi|32966037|gb|AY327547.1	AATACAGCGACAACAATAAT	49.993	GTAGGAAGGTCGGTATTTAT	50.213	138
gi|158832352|dbj|AB300577.1	AGAATTTGGCATCTGGT	50.834	GCGCTCTCTCTCTCTCT	49.994	126
gi|30961873|gb|AY283926.1	ATCTGGTGCATACTACTTGT	49.551	GTTGGGAGCTCTAGTGA	48.573	170
gi|30961871|gb|AY283924.1	CATGATAAAGTAGGGTTTTG	50.01	AAATAAGCTTAGTATTTGCG	49.769	174

Contd...

Table 3: Contd...

Contigs	Forward primer	Temperature forward (°C)	Reverse primer	Temperature reverse (°C)	Product size (bp)
gi|30961868|gb|AY283921.1	TTATGAGTTCGATACGTAGG	50.531	GAAGAGTGAGTGTGTTTCTC	49.306	156
gi|30961872|gb|AY283925.1	TTAGAAAAGTGGAGCAGTAG	50.008	TAAAACGACACCTTTTTAAT	49.576	167
gi|30961870|gb|AY283923.1	GACTGAGTAAGCACACAGAT	49.938	CTCCAGCTGTAATCTTCATA	50.503	120

SSRs: Simple sequence repeats

typhoid fever, and tuberculosis.[19] The SSR-based primers/markers have an extensive application in plant genetics and breeding. Hence, in our study, the potential SSR-based forward and reverse primers were designed for 59 out of 108 nucleotide sequences of the *Acanthaceae* species including *Avicennia germinans*, *Avicennia alba*, *Blepharis subvolubilis*, *Ruellia ciliatiflora*, *Ruellia nitida*, *Ruellia eurycodon*, *Ruellia pedunculosa*, *Aphanosperma sinaloensis*, *Kalbreyeriella rostellata*, *Aphelandra verticillata*, *A. paniculata*, and *A. ilicifolius*.

CONCLUSION

Microsatellites or SSRs play a major role in polymorphism analysis and in marker assisted selection. *In silico* approach for predicting SSRs in the whole genome, was found to be both cost and time effective and also helps to develop a new generation of molecular markers as well. In our study, the microsatellites and its associated primers were identified for the publically available *Acanthaceae* family genomes using computational methods. The identified microsatellites and markers might pave the way for further studies in the aspect of breeding and genetic studies of the plants that belong to the family *Acanthaceae*.

REFERENCES

1. Alam AH, Rashid MH, Rahman MA, Baki MA, Sadik MG. Chemical constituents of *Hemigraphis hirta* T. Anders. (Acanthaceae). Pak J Biol Sci 2002;5:1264-6.
2. Bhatt A, Naidoo Y, Nicholas A. The foliar trichomes of *Hypoestes aristata* (Vahl) Sol. ex Roem. and *Schult* var *aristata* (Acanthaceae) a widespread medicinal plant species in tropical sub-Saharan Africa: With comments on its possible phylogenetic significance. Biol Res 2010;43:403-9.
3. Islam MT, Das PR, Kabir MH, Akter S, Khatun Z, Haque MM, *et al*. Acanthaceae and *Asteraceae* family plants used by folk medicinal practitioners for treatment of malaria in Chittagong and Sylhet Divisions of Bangladesh. Am Eurasian J Sustain Agric 2012;6:146-52.
4. Sawadago WR, Meda A, Lamien CE, Kiendrebeogo M, Guissou PI, Nacoulma OG. Phenolic content and antioxidant activity of six *Acanthaceae* for Burkina Faso. Asian Netw Sci Inf 2006;6:249-52.
5. Li C, Zhang Y, Ying K, Liang X, Han B. Sequence variations of simple sequence repeats on chromosome-4 in two subspecies of the Asian cultivated rice. Theor Appl Genet 2004;108:392-400.
6. Sathishkumar R, Lakshmi PT, Annamalai A, Arunachalam V. Mining of simple sequence repeats in the Genome of Gentianaceae. Pharmacognosy Res 2011;3:19-29.
7. Gupta PK, Varshney RK. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 2000;113:163-85.
8. Singh S, Gupta S, Mani A, Chaturvedi A. Mining and gene ontology based annotation of SSR markers from expressed sequence tags of *Humulus lupulus*. Bioinformation 2012;8:114-22.
9. Gupta S, Shukla R, Roy S, Sen N, Sharma A. *In silico* SSR and FDM analysis through EST sequences in *Ocimum basilicum*. Plant Omics J 2010;3:121-8.
10. Rajendrakumar P, Biswal AK, Balachandran SM, Srinivasarao K, Sundaram RM. Simple sequence repeats in organellar genomes of rice: Frequency and distribution in genic and intergenic regions. Bioinformatics 2007;23:1-4.
11. Li AQ, Zhao CZ, Wang XJ, Liu ZJ, Zhang LF, Song GQ, *et al*. Identification of SSR markers using soybean (*Glycine max*) ESTs from globular stage embryos. Electron J Biotechnol 2010;13:1-11.
12. Available from: http://www.ncbi.nlm.nih.gov/nuccore/? term= Acanthaceae. [Last accessed on 2013 Jun 10].
13. Available from: http://www.minerva.ufpel.edu.br/~lmaia.faem/. [Last accessed on 2013 Aug 05]
14. Da Maia LC, Palmieri DA, de Souza VQ, Kopp MM, De Carvalho FI, Costa de Oliveira A. SSR Locator: Tool for simple sequence repeat discovery integrated with primer design and PCR simulation. Int J Plant Genomics 2008;2008:412696.
15. Tóth G, Gáspári Z, Jurka J. Microsatellites in different eukaryotic genomes: Survey and analysis. Genome Res 2000;10:967-81.
16. Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R. Computational and experimental characterization of physically clustered simple sequence repeats in plants. Genetics 2000;156:847-54.
17. Yuan D, Liang S, Lin Z, Zhang X. *In silico* comparative analysis of EST-SSRs in three cotton genomes. Afr J Biotechnol 2012;11:13269-371.
18. Soumyanath A. Traditional Medicines for Modern Times: Antidiabetic Plants. USA: CRC Taylor and Francis Publication; 2006. p. 49.
19. Nabere O, Adama H, Samson G, Kiessoum K, Patrice Z, Roland MN, *et al*. Antibacterial and phytochemical studies of three *Acanthaceae* species used in Burkina Faso traditional medicine. J Appl Pharm Sci 2013;3:49-55.

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