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Mining of microsatellites in mitochondrial genomes of order Hypnales (Bryopsida)

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Abstract

Microsatellites or SSRs are the markers of selection due to their reproducibility, degree of polymorphism, distribution throughout the genome and co-dominant nature. Microsatellites are used primarily to study the genetic variability in various species and marker aided selection. Since microsatellites can be readily amplified by PCR, they have been utilized most extensively. To reduce time and cost to a great extent, the computational approach for identifying and developing microsatellite markers by mining nucleotide sequences is preferred over the conventional methods. In the present analysis, an *in-silico* method was used to detect microsatellites effectively in mitochondrial genomes of *Anomodon rugelii* (Müll. Hal.) Keissl., *Anomodon attenuatus* (Hedw.) Hueb., *Climacium americanum* (Renauld & Cardot) Kindberg, and *Hypnum imponens* Hedw. (Bryopsida; Hypnales). A total of 101 perfect microsatellites were mined with an average density of 1 microsatellite/4.21 kb. The hexa-nucleotide repeats were not detected in mitochondrial genomes of studied taxa. Di-nucleotides were seen to be the most frequent repeats followed by tetra-nucleotides. The identified microsatellites were also checked for variability in length between species. The mined microsatellites will be used for gene tagging, species identification and population genetic studies.

Keywords: Bryophytes; Hypnales; Microsatellites; Simple Sequence Repeats

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Introduction

Microsatellites, which is also acknowledged as Simple Sequence Repeats (SSRs) are short repeat sequences of 1-6 nucleotides present in both prokaryotic and eukaryotic organism (1, 2). These repetitions are omnipresent and play a key role in the development of molecular markers. The traditional methods for producing microsatellites markers are usually laborious whereas these can be quickly identified using nucleotide sequence data through computational methods (3, 4). Computational microsatellites mining is easier, inexpensive and helps in repeat identification in whole genome sequences. Therefore, microsatellites specific biological databases were developed (1, 5, 6).

Bryophytes (liverworts, mosses and hornworts) hold the basal position in land plants

and play a major role in diverse terrestrial ecosystem (7). In chloroplast genome sequences of Bryophytes microsatellites were identified (8). However, there is scarcity of such studies for mitochondrial genomes of plants because in comparison with chloroplast genome sequences very few mitochondrial genomes have been sequenced in plant Kingdom which includes algae, bryophytes, pteridophytes, gymnosperms and angiosperms (9, 10). Earlier, microsatellites were detected in mitochondrial genomes of two mosses (*Physcomitrella patens* and *Anomodon rugelii*), two liverworts (*Marchantia polymorpha* and *Pleurozia purpurea*) and two hornworts (*Phaeoceros laevis* and *Nothoceros aenigmaticus*) (11). In the present study, mitochondrial genomes of 4 taxa under order Hypnales were used to mine for microsatellites and analyse their distribution.

Materials and Methods

Retrieval of mitochondrial genome sequences

Sequences of mitochondrial genomes of order Hypnales available at National Center for Biotechnology Information (NCBI) were retrieved in FASTA format (Table 1).

Table 1. List of studied organisms

Sl. No.	Organism	Accession	Size (kb)	Ref.
1	*Anomodon rugelii*	NC_016121.1	104.239	(15)
2	*Anomodon attenuatus*	NC_021931.1	104.252	(16)
3	*Climacium americanum*	NC_024515.1	105.048	(16)
4	*Hypnum imponens*	NC_024516.1	103.83	(16)

Mining of microsatellites

For the identification of microsatellites in mitochondrial genomes of order Hypnales, MISA (a Perl program) was used. MISA generates information of perfect microsatellites by taking FASTA file as an input. To mine the microsatellite, the minimum repeat size of ≥12 for mono-, ≥6 for di-, ≥4 for tri-, ≥3 for tetra-, penta- and hexa-nucleotides was used. Interruption between two microsatellites was taken as 0.

Identification of common, polymorphic and unique microsatellites

Basic Local Alignment Search Tool (BLAST) was used to classify common, polymorphic and unique microsatellites showing variability in length. Similarity of microsatellites flanking regions between different species was used to detect homologous relationship as used earlier (1, 12).

Results and Discussion

Screening of mitochondrial genome sequences of order Hypnales for microsatellites mining

A total of 101 perfect microsatellites were mined with an average density of 1 microsatellite/4.21 kb in mitochondrial genome sequences of order Hypnales (*A. attenuatus*, *A. rugelii*, *C. americanum* and *H. imponens*). In this study, di-nucleotide (51, 50.5%) motifs were most frequent followed by tetra- (26, 25.7%), tri- (15, 14.9%), mono- (7, 6.9%) and penta-nucleotide repeats (2, 1.9%), whereas hexa-nucleotide repeats were completely absent (Fig. 1). Frequency of identified repeats in order Hypnales is presented in Table 2.

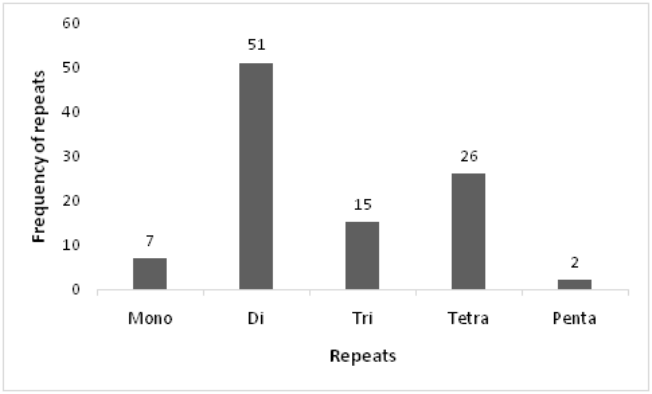

Fig. 1. Identified repeats in mitochondrial genomes of order Hypnales

The average density of microsatellites identified in this study was higher with earlier study performed on Magnoliids (1 microsatellite/6.91 kb) (2), however, lower than *Arabidopsis* (1 microsatellite/0.33 kb) (12), and Solanaceae family (1 microsatellite/1.26 kb) (13).

In *A. rugelii*, a total of 22 microsatellites were identified with a density of 1 microsatellite/4.74 kb sequence mined. Among these, di-nucleotide (10) was most frequent followed by tetra- (7), tri- (4) and mono-nucleotide (1), while penta- and hexa-nucleotide was absent in this genome. Interestingly, frequency and distribution of microsatellites identified in *A. attenuatus* was identical with *A. rugelii*. This is due to the conservation of mitochondrial genomes (4, 9). Frequency of motifs identified in *A. rugelii* and *A. attenuatus* is presented in Table 3.

In *C. americanum*, a total of 30 microsatellites were mined with a density of 1 microsatellite/3.5 kb sequence. Di-nucleotide (16) was most frequent followed by tetra- (6), tri- (4), mono- (3), and penta-nucleotide (1) repeats. Frequency of motifs identified in *C. americanum* is presented in Table 3.

A total 27 microsatellites were found with a density of 1 microsatellite/3.85 kb sequence in *H. imponens*. Among these, Di-nucleotide (15) was most frequent followed by tetra- (6), tri- (3), mono- (2), and penta-nucleotide (1) repeats. Frequency of motifs identified in the selected species is presented in Table 3.

Common, polymorphic and unique microsatellites

The common and polymorphic microsatellites were also observed between each pair of organisms. The range of common and

Table 2. Frequency of various repeats detected in mitochondrial genomes of order Hypnales

Sl. No.	Organism	Mono	Di	Tri	Tetra	Penta	Total	Density
1	*A. rugelii*	1	10	4	7	-	22	4.73814
2	*A. attenuatus*	1	10	4	7	-	22	4.73873
3	*C. americanum*	3	16	4	6	1	30	3.5016
4	*H. imponens*	2	15	3	6	1	27	3.84556
Total		**7**	**51**	**15**	**26**	**2**	**101**	

Table 3. Distribution of microsatellite motifs in both species of *Anomodon*, *C. americanum* and *H. imponens*

Sl. No.	Motif	Frequency		
		A. rugelii* & *A. attenuatus	***C. americanum***	***H. imponens***
1.	A	1	2	2
2.	AAT	1	1	1
3.	AATC	1	1	–
4.	AT	7	12	13
5.	ATAA	2	1	2
6.	ATAG	1	1	1
7.	ATTT	1	1	1
8.	CT	1	1	1
9.	GAA	3	2	2
10.	TA	2	3	1
11.	TAGA	1	1	1
12.	TTTA	1	1	1
13.	G	–	1	–
14.	TGGGG	–	1	–
15.	TTC	–	1	–
16.	TAGAA	–	–	1
		Total=22	Total=30	Total=27

Table 4. Common, polymorphic and unique microsatellites identified in mitochondrial genomes of order Hypnales

	Unique	***A. rugelii***	***A. attenuatus***	***C. americanum***	***H. imponens***
A. rugelii	-	-	**21**	**16**	**17**
A. attenuatus	-	1	-	**17**	**17**
C. americanum	*5*	3	2	-	**17**
H. imponens	*3*	2	2	6	-

Note: Bold: Common, Underlined: polymorphic, Italicized: unique

polymorphic microsatellite was from 16 to 21 and 1 to 6 respectively. Similarly, in a recent study length variation of microsatellites between each pair of species was identified in the genus *Arabidopsis* (12) and *Triticum* (14). Frequency of common, polymorphic and unique microsatellites identified is presented in Table 4.

Conclusion

Microsatellites were successfully identified in mitochondrial genomes of order Hypnales. These repeats will be most commonly used in various research fields, including genetic diversity analysis, gene mapping, species identification and assisted selection of markers. In addition, they can be further used in phylogenetic and diversity studies of order Hypnales.

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Authors' contributions

AS designed the study framework and help in the analysis. KA mined all the data and compiled the draft of manuscript. SK helps in data analysis and preparation of manuscript. AA provided all the needful related to the species studied under order Hypnales of class Bryopsida.

Conflict of interest

Authors declare no conflict of interest.

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