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Potential of Microsatellites Markers for the Genetic Analysis of Bryophytes

Saumy PANDEY, Vinay SHARMA, Afroz ALAM*

*Banasthali University, Departament of Bioscience and Biotechnology, Banasthali 304022, Rajasthan India; saumbhavi@gmail.com; vinaysharma30@yahoo.co.uk; afrozalamsafvi@gmail.com (*corresponding author)*

Abstract

Microsatellites have increasingly being used to study genetic diversity, phylogeny, population genetics, population ecology and genetic mapping of bryophytes. Due to co-dominant and highly reproducible features, microsatellites became markers of choice for several genetic analyses of bryophytes. However, the major limitation is *de novo* isolation of microsatellites from the interest species which were studied and gave genomic libraries. Initially, traditional methods of microsatellite development were tedious and time consuming, but due to the sequencing of several bryophytes available in public databases, advancement in PCR technologies and computer software, have cumulatively facilitated the development of microsatellites for bryophytes study. This review examines the features, strategies for the development of microsatellites and their utilization in many aspects of genetic and ecological studies of bryophytes.

Keywords: DNA polymorphism, genetic diversity, genome sequences, molecular markers, microsatellites, moss

Introduction

Molecular markers are powerful tools for studying the genetic biodiversity, as these markers show Mendelian inheritance, making it possible to trace the fingerprint of each organism and determine the evolutionary history of the species by phylogenetic analysis, studies of genetic relationship, population genetic structures and genetic mapping. Hamada *et al.* (1982) first showed the existence of microsatellites in eukaryotic genomes, while Litt and Luty (1989) used the term "microsatellites" during their study on cardiac gene.

Bryophytes significantly contribute to the biodiversity of terrestrial ecosystems. However, the bryodiversity studies based on morphological features are often hindered by the unclear species circumscription, identification difficulties of bryophyte taxa and the influence of the environment in the evolution of those characters. Currently, DNA sequence analyses provide new tools for the study of diversity within and among species (Chakravarthi and Naravaneni, 2006; Jonah *et al.*, 2011). Several studies have revealed the difference in information provided by the morphological and molecular data, supporting the relevance of molecular markers (Zouhair *et al.*, 2000; Sotiaux *et al.*, 2009; Shaw, 2009; Vanderpoorten and Shaw, 2010). Thus, it might be said that molecular data provide a more accurate representation of phylogenetic history and relationships than morphological characters alone (Holyoak and Pedersen, 2007).

Because of the presence of both highly conserved and variable regions, Restriction Fragment Length Polymorphism (RFLP) (Boisselier-Dubayle *et al.*, 1995a; Patterson *et al.*, 1998), Random Amplified Polymorphic DNA (RAPD) (Boisselier-Dubayle *et al.*, 1995a, 1995b; Skotnicki *et al.*, 1998a; Wolfe and Liston, 1998; Korpelainen and Allen, 1999) and microsatellites or Simple Sequence Repeats (SSRs) (Becker and Heun, 1994) have been used to reveal the genetic relationship among different taxa of bryophytes. Of all these techniques that facilitate the evaluation of genetic diversity, microsatellites or SSR are preferred, since it would make possible to detect in a simple manner, a large number of DNA polymorphism (Park *et al.*, 2009).

Microsatellites characteristics

Microsatellites (Litt and Luty, 1989) also known as Simple Sequence Repeats (SSRs) (Tautz *et al.*, 1986; Jacob *et al.*, 1991), Short Tandem Repeats (STRs) (Edwards *et al.*, 1991) or Simple Sequence Length Polymorphism (SSLPs) (McDonald and Potts, 1997), are tandem repeats of 1-6 nucleotides (Gupta *et al.*, 1996; Thiel *et al.*, 2003) that mutate frequently as compared to other genomic regions and hence show high levels of genetic variation (Farooq and Azam, 2002). The variation of the tandemly repeated units is mainly due to strand slippage during DNA replication (Levinson and Gutman, 1987), where the repeats allow a new matching via excision or addition of repeats (Schlotterer and Tautz, 1992). As the probability of strand slippage during replication is greater than point mutations, the microsatellite loci tends to be hyper-variable. Microsatellite assays demonstrate extensive inter-individual length polymorphisms, within the employment of specific primer sets during PCR analysis, thus with unique loci using discriminatory primer sets.

Microsatellites can be classified on the basis of repeated sequences as (a) perfect repeats: they have only perfect repetitions of nucleotide sequences, e.g. $(GC)_{10}$; (b) imperfect repeats: have repeated sequences that are interrupted by different nucleotides that are not repeated, e.g. $(GC)_{10}AT(GC)_{10}$; (c) composite

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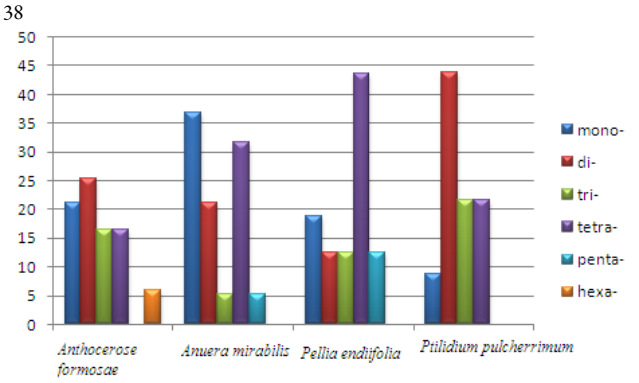

Fig. 1. The frequency of each SSR repeats (mono- to hexa) in four bryophytes species (Shanker 2013a, 2013b, 2014a, 2014b)

repeats: have two or more different motifs in tandem, e.g. $(GC)_7(AT)_6$. The composite repeats can be perfect or imperfect. Mononucleotide repeats face problems during PCR amplification, whereas di-, tri- and tetranucleotide repeats sequences are used for the majority of the molecular genetic studies (Selkoe and Toonen, 2006). Dinucleotide repeats account for the majority of microsatellites for several species (Li *et al*., 2002). A trinucleotide and hexanucleotide repeat usually occurs in the coding regions as they do not cause a frame-shift (Toth *et al*., 2000). Microsatellites with longer repeats are less common and data regarding their evolution is limited (Li *et al*., 2002). The level of inter- and intraspecific polymorphism is higher when the tandem repeats are greater (Queller *et al*., 1993). The abundance of one particular repeat unit of a nucleotide in SSR motifs of a chloroplast genome sequence of four different genera of bryophytes is shown in Fig. 1.

Microsatellites can also be present in organelle genomes such as those of mitochondria and chloroplast. The chloroplast and mitochondrial genomes usually have an uniparental mode of transmission, so they display different patterns of genetic differentiation compared to nuclear alleles (Provan *et al*., 1999a, 1999b). Thus, for a complete understanding of plant genetic variation and evolution, all three genomes (nuclear, chloroplast and mitochondria) must be considered; therefore, in addition to nuclear microsatellites, marker techniques based on the chloroplast and mitochondrial microsatellites have also been developed (Agrawal *et al*., 2008). The complete genome sequence of mitochondrial and chloroplast genome is available for several species of bryophytes (Table 1), thus several microsatellites markers have been developed for bryophytes (Zhao *et al*., 2014; Shanker, 2013a, 2013b, Shanker 2014) and utilized for studding their genetic diversity, population ecology, phylogeny and evolution study.

Useful characteristics of microsatellites in the study of bryophytes:

1. Co-dominance: The co-dominant genetics of microsatellites offer a major advantage over other fingerprinting approaches such as RAPDs, AFLPs and ISSRs, especially for studies of hybridization and mating patterns, since both parental genomes can be detected directly by PCR amplification.

2. High abundance: Microsatellites are present in both coding and non-coding regions (Tautz and Renz, 1984; Gupta *et al*., 1994; Toth *et al*., 2000) with higher density in the non-coding regions of eukaryotes (Hancock, 1995; Li *et al*., 2002). Microsatellites are found to be dispersed in diverse genomic regions, including 3′-UTRs, 5′-UTRs, exons and introns (Rajendrakumar *et al*., 2007). In plants, SSRs are much more abundant and preferentially associated within untranslated regions (UTRs) of the transcribed regions (Morgante *et al*., 2002).

3. High allelic diversity: Microsatellite markers have high rates of mutation (on average 5×10^{-4} mutation per locus per generation) thus resulting in high levels of allelic diversity.

4. High reproducibility: Microsatellites are highly reproducible and produce consistent data when used by different research laboratories (Saghai-Maroof *et al*., 1984). Also, lengthy primers and high annealing temperatures enhance the reproducibility during genotyping.

5. Transferability: Microsatellites are transferable, because their flanking regions are highly conserved across taxa, allowing cross-species amplification. The transferability of SSRs derived from EST databases (EST-SSR) is greater than that of SSRs derived from enriched genomic DNA libraries. The EST-SSRs originate from expressed regions, and therefore they are more conserved across a number of related species than non-coding regions (Varshney *et al*., 2005).

6. Microsatellites require a low quantity of template DNA: As SSR is PCR based techniques, the quantity of DNA required for SSR-PCR fingerprinting is very low (Kumar *et al*., 2009; Wolko *et al*., 2010).

Development of microsatellites

In spite of the wide applicability of microsatellite markers in bryodiversity studies, the number of microsatellite marker developed for bryophytes is very deficient (Provan and Wilson, 2007; Hutsemékers *et al*., 2008; Liu *et al*., 2010; Sawicki *et al*., 2012). Because the designing of primers, sequence information is required, thus the microsatellites have to be isolated *de novo* from the species studied for the first time. As the frequency of microsatellites in plants' genome is relatively less compared to animals' genome, it causes problems with their large scale isolation (Powell *et al*., 1996). Traditionally, microsatellite loci were isolated from partial genomic libraries of the species of interest by screening several thousands of clones through colony hybridization. This method is simple, but inefficient for species with low microsatellite frequencies (Zane *et al*., 2002). Conventional genomic library construction and subsequent screening are time consuming, tedious, costly and require high level of expertise. Even more, AT dinucleotides, which are the most abundant type of SSR in plants, are difficult to isolate from libraries because they are palindromic (Powell *et al*., 1996). Therefore, several alternative methods have been developed in order to reduce the time invested in microsatellite isolation and to significantly increase the yield of microsatellite loci. These methods involve database mining, transferability of markers and sequencing.

Development of microsatellite through genomic library construction

For microsatellite loci isolation from genomic libraries of interest species several methods have been developed which include selective hybridization (Karagyozov *et al*., 1993; Armour *et al*., 1994; Kandpal *et al*., 1994; Hamilton *et al*., 1999), primer extension enrichment (Ostrander *et al*., 1992; Paetkau, 1999) and several other methods which have been

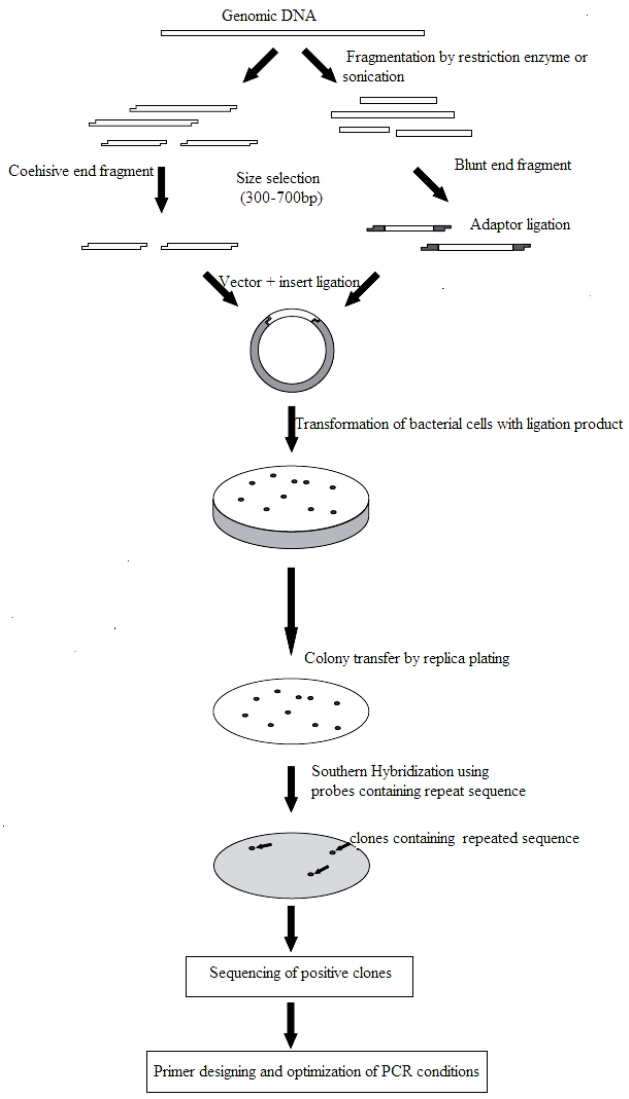

Fig. 2. Protocol for the development of SSR markers through SSR-enrichment method (modified scheme from Zane *et al*., 2002)

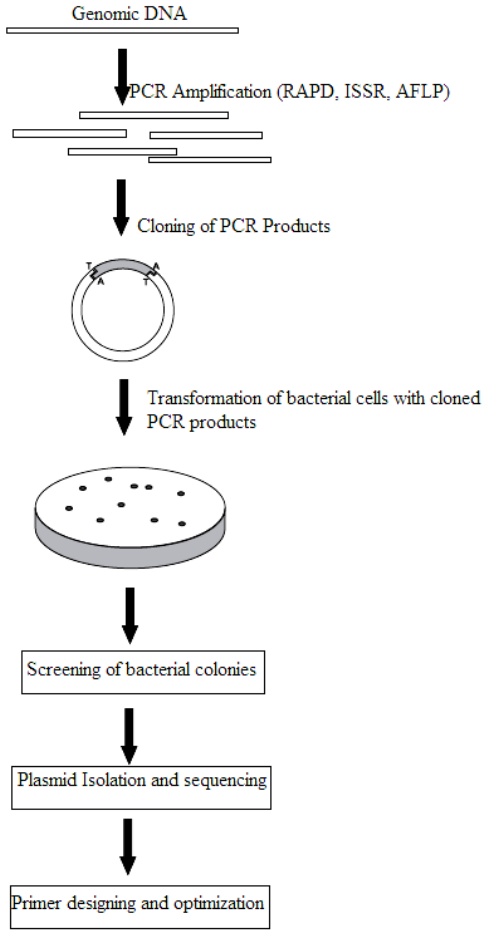

Fig. 3. Protocol for the development of SSR markers through cloned PCR products (modified scheme from Zane *et al*., 2002)

reviewed extensively (Zane *et al*., 2002; Weising *et al*., 2005; Kalia *et al*., 2011). Hutsemékers *et al*. (2008) identified 8 nuclear microsatellite loci in the aquatic moss *Platyhypnidium riparioides* using the microsatellite-enriched library's method. The markers amplified 3-7 alleles per locus and can further be used to investigate the diversity and population genetic structure.

Microsatellite isolation through genomic library construction is very tedious and time consuming and, it is not recommended for the taxa containing a low frequency of microsatellites, such as plants, or when a large number of microsatellites are required, as in the case of studies on genetic distances among populations (Zhivotovsky and Feldman, 1995; Cooper *et al*., 1999) or when constructing a genetic map (Liu, 1997).

A general protocol for the development of SSR markers through SSR-enrichment method is described in Fig. 2 (modified scheme from Zane *et al*., 2002).

Microsatellite markers can be developed by cloning PCR products generated from RAPD primers, ISSR primers, AFLP or 5' anchored microsatellite primers. Liu *et al*. (2010) used FIASCO (FAST isolation of AFLP sequence containing repeats) protocol (Zane *et al*., 2002) to develop 13 microsatellite primers for *Ptychomitrium gardneri*. Out of which 8 primer pairs produced polymorphic products. These markers amplified three to four alleles per locus. Cross amplification of these primers was tested on four *Polytrichum* species (*P. linearifolium, P. wilsonii, P. fauriei* and *P. sinense*) whereas 5 primer pairs amplified in *P. linearifolium* and *P. wilsonii*. ISSR cloning method was used by Provan and Wilson (2007) to develop 9 microsatellites for the moss species *Sphagnum capillifolium*, which amplified 3-7 alleles per locus and also exhibited cross species amplification. A general protocol for the development of SSR markers through cloned PCR products is described in Fig. 3 (modified scheme from Zane *et al*., 2002).

Database mining

Currently, microsatellite markers are developed by screening the already submitted sequence information of ESTs, cDNA and fully sequenced genes in a public database such as EMBL, GenBank, or DNA Data Bank of Japan (DDBJ), for the presence of microsatellite in the nuclear genome or within the organelle genome. Initially, unspecific alignment tools such as BLASTN (Altschul *et al*., 1990) were used for database searches. Now days, a number of web based SSR search

Table 1. The complete genome sequence of mitochondrial and chloroplast genomes of bryophytes submitted at NCBI

S.No.	Organellar genome	Species	Genome size (bp)	Reference
		Liverworts		
1		*Aneura mirabilis*	108007	Wickett *et al*., 2008
2		*Marchantia polymorpha*	121024	Ohyama *et al.*, 1986
3		*Ptilidium pulcherrimum*	119007	Forrest *et al*., 2011
		Mosses		
4	Chloroplast genome	*Physcomitrella patens*	122890	Sugiura *et al*., 2003
5		*Tortula ruralis*	122630	Oliver *et al*., 2010
		Hornworts		
6		*Anthoceros formosae*	161162	Kugita *et al*., 2003
		Liverworts		
7		*Marchantia polymorpha*	186608	Oda *et al*., 1992
8		*Pleurozia purpurea*	168526	Wang *et al*., 2009b
9		*Treubia lacunosa*	151983	Liu *et al*., 2011
		Mosses		
10	Mitochondrial genome	*Physcomitrella patens*	105340	Terasawa *et al*., 2007
11		*Anomodon rugelii*	104239	Liu *et al*., 2011
		Hornworts		
12		*Phaeoceros laevis*	209482	Xue *et al*., 2010
13		*Megaceros aenigmaticus*	184908	Li *et al*., 2009

Table 2. Total number of microsatellite loci identified by screening mitochondrial genome sequence of bryophytes (Zhao *et al*., 2014)

S. No.	Species	Total no. of microsatellite loci
	Liverwort	
1	*Marchantia polymorpha*	88
2	*Pleurozia purpurea*	69
	Mosses	
3	*Physcomitrella patens*	83
4	*Anomodon rugelii*	59
	Hornworts	
5	*Phaeoceros laevis*	55
6	*Nothoceros aenigmaticus*	69

Table 3. Total number of microsatellites identified by screening chloroplast genome sequence of bryophytes (Shanker *et al*., 2013a, 2013b, 2014a, 2014b)

S.No.	Species	Total no. of microsatellites
1	*Anthoceros formosae*	67
2	*Aneura mirabilis*	19
3	*Pellia endiviifolia*	16
4	*Ptilidium pulcherrimum*	23

software such as MISA, SSR locator, CUGssr, Sputnik and SSRSEARCH are used for screening and hence for the development of SSR markers for different species. The microsatellite markers derived from EST sequence are more useful when compared to markers derived from anonymous regions (Varshney *et al*., 2005; Kashi and King, 2006; Varshney *et al*., 2006). EST-SSRs were derived from several species of bryophytes such as *Marchantia polymorpha, Synchtria ruralis* and *Physcomitrella patens* (Victoria *et al*., 2011). Shanker (2014b) designed 22 SSR primers from 23 CpSSR, by screening the chloroplast genome sequence of *Ptilidium pulcherrimum.*

The full genome sequences of chloroplast and mitochondrial genomes (Table 1) are available for many species of bryophytes, thus several microsatellite markers have been developed for bryophytes screening.

Using database search method, Zhao *et al*. (2014) and Shanker (2013a, b) screened the mitochondrial and chloroplast genome sequences of some bryophytes species submitted in NCBI for the presence of microsatellites loci using MISA software. The number of microsatellites identified is shown in Tables 2 and 3 respectively. Further, these microsatellites regions identified by database mining can be used for designing primers for specific plant groups, which can also be used in the genetic diversity study of related species, due to the transferability of SSR primers.

Next generation sequencing

The chloroplast genome sequence of leafy liverwort, *Ptilidium pulcherrimum*, was sequenced using next generation sequencing (Forrest *et al*., 2011). *P. pulcherrimum* was the first bryophyte plant to be sequenced using this technology. Sawicki *et al*. (2012) used next generation sequencing technology to develop 46 microsatellite primer pairs for *Orthotrichum speciosum*. Out of 92 SSR motifs identified in 89 countings, only 46 had flanking regions suitable for primer design. These 46 primer pairs were tested on 40 individuals of *Orthotrichum speciosum* collected from 2 populations, revealing 35 polymorphic loci. The designed primer showed transferability of phylogenetically closely related *species O. affine* and *O. striatum*, and distantly related, *O. diaphanum* and *O. pallens.*

Microsatellites points of issue

In spite of their recognised advantages that microsatellites offer within the genetic analysis, there are few limitations or drawbacks associated with this technique that might affect data analysis (Bonin *et al.,* 2004). Many limitations of microsatellites marker can be avoided by a careful selection of microsatellite loci during the isolation process (Selkoe and Toonen, 2006).

Homoplasy: referring to alleles similar in size, but with different lineages (Jarne and Lagoda, 1996). Due to the homoplasy, the actual allelic diversity between populations is underestimated (Estoup *et al*., 1995; Jarne *et al*., 1998; Curtu *et al*., 2004). Homoplasy is usually common in compound or interrupted repeats (Adams *et al*., 2004) and it can be categorized in two groups (a)

detectable homoplasy, (b) non detectable homoplasy. The detectable homoplasy can be revealed by nucleotide sequencing. The detectable homoplasy only accounts for only 1-2% for the underestimation of allelic diversity (Adams *et al*., 2004; Curtu *et al*., 2004).

In general, homoplasy is less problematic in population genetic analysis, since the chance of homoplasy is proportional to the genetic distance of two individuals or populations (Estoup *et al*., 2002). However, it creates problems during studies involving highly divergent groups, such as for phylogenetic reconstruction (Estoup *et al*., 1995).

Null alleles: Sometimes the absence of PCR products is not due to the failure of PCR reaction, but due to the presence of null alleles at the SSR locus. Null alleles arise due to the mutation at primer annealing site and thus prevents the locus amplification (Paetkau and Strobeck, 1995). Dakin and Avis (2004) study showed that a low rate of null alleles may have a negligible effect on most population analysis, but have considerable impact on the parentage analysis. Thus, for consistent amplification it is advised that primer selection should be done carefully before large scale sample analysis.

Shutter bands: Strand slippage during PCR amplification produce shutter bands (Hauge *et al*., 1993; Ellegren, 2004) that vary in size from the main product by multiples of the length of repeat units (Hauge *et al*., 1993; Murray *et al*., 1993; Smulders *et al*., 1997). Since the Taq polymerase slippage is directly proportional to the number of repeat units and inversely proportional to the length of the repeat unit, the occurrence of shutter bands will be prominent in SSRs with long stretches of a short repeat unit (1-2bp) (Shinde *et al*., 2003).

Microsatellites applications

Microsatellites emerge the opportunity to study genetic diversity, population genetics, reconstructing the phylogenetic relationship among and within species, population ecology, biogeography, paternity and ploidy of plants (Arroyo-Garcia *et al*., 2002; Rajendrakumar *et al*., 2007; Magain *et al*., 2010).

Genetic diversity and taxonomy

Genetic diversity is defined as the variation in nucleotides, genes, chromosomes or whole genomes of organisms (Wang *et al*., 2009a). Microsatellite analysis of several species of bryophytes exhibit high level of genetic diversity (Wilson and Provan, 2003; Shaw *et al*., 2008; Hutsemekers *et al*., 2010), while before these methods it was assumed that bryophytes, having haploid gametophytes, retain a low level of genetic variation due to natural selection. The high level of genetic diversity in bryophytes may be due to multiple-niche selections (Wyatt *et al*., 1989), inter-locus interaction, e.g. epistasis (Shaw and Beer 1999), sexual reproduction (Wyatt *et al*., 2005) and somatic mutations (Skotnicki *et al*., 2005). Paasch *et al*. (2015) demonstrated that the high level of genetic diversity in the xeric populations of *Syntrichia caninervis* is mainly due to migration and somatic mutation.

Kophimai *et al*. (2014) studied genetic diversity in two closely related moss species *Scorpidium cossonii* and *Scorpidium revolvens* respectively, using nine microsatellite markers and concluded that *Scorpidium cossonii* is genetically more diverse than *Scorpidium revolvens* due to different mating systems, distinct population sizes and different population histories.

Molecular analysis of species provides more accurate information about phylogeny and relationships than molecular characters based analysis. Thus, molecular phylogenetics has gained importance in testing traditional taxonomic hypotheses, especially in taxa with reduced morphologies like bryophytes. In bryophytes, the main reasons for the difficulties in morphological and molecular based analysis of species are the limited characters defining them, the focus on a few key-characters and morphological plasticity due to environment (Vanderpoorten and Goffinet, 2006). Several deviation patterns between morpho-species concepts and molecular phylogenies have been reported (Heinrichs *et al*., 2009a). Generally, the sequence related markers have been used for molecular phylogenetic studies (Samigullin *et al*., 1998; Olsson *et al*., 2009; Bell and Hyvönen, 2010; Merget and Wolf, 2010). Few reports suggest the use of microsatellite markers for the phylogenetic studies. Ramaiya *et al*. (2010) studied *Frullania* sp. sampled from North Carolina using nucleotide sequence (trnL, trn F and ITS region); the results revealed no variation and no phylogenetic structure within Eastern North-American species. However, variation at 12 hypervariable microsatellite loci revealed two well defined groups of populations. Also the microsatellite analysis presented these two groups of the population as reproductively isolated biological species.

The genetic structure of the *Sphagnum warnstorfii* population shows the partial correlation with pH, but independence of the geographic position. Microsatellite markers are also used to study reproductive biology and its effect on genetic variation and genetic structure within populations of several species of bryophytes (Vander-Velde *et al*., 2000; Vander-Velde *et al*., 2001a; 2001 b; Leonardia *et al*., 2012). The occurrence of maternal paternity (polyandry) in moss species *Sphagnum lescurii* (Szovenyi *et al*., 2009b) was also studied using microsatellite.

Population ecology

SSR markers are used to investigate the population genetic structure at different spatial scale, the distribution of genetic variation and the level of gene flow within the population. In bryophytes, genetic dispersal occurs mainly through spores, sperms and vegetative fragments. Thus, gene flow can be limited and genetic isolation by distance occurs within populations (Wright, 1943; Vekemans and Hardy, 2004). The nature of gene flow within population not only affects the genetic structure of population, but also the ability of local adaptation of a population which may result in an independent evolution of the populations, thus causing speciation (Slatkin, 1985). In bryophytes, there are only a few studies that test the relation between genetic structure and environmental factors (Szovenyi *et al*., 2009a; Hutsemekers, 2010; Karlin *et al*., 2011a; Johnson, 2012; Szovenyi *et al*., 2012). Mikulaskova *et al*. (2015) used 12 microsatellite loci analysis to reveal the relation between *Sphagnum warnstorfii* genetic variability within different populations and pH/calcium gradient in central Europe.

Habitat fragmentation has adverse effects on the genetic biodiversity because the decrease in the level of gene flows. The habitat fragmentation due to harvesting of peat moss (*Polytrichum commune*) has been studied using microsatellites (Wilson and Provan, 2003). The authors reported the deleterious effect of habitat fragmentation on the genetic diversity due to the process of genetic drift (Wilson and Provan, 2003; Leonardia, 2012). However, the value of genetic diversity obtained for *Polytrichum formosum* population using microsatellite was higher than the one obtained using allozymes. Further, the value of genetic diversity (H) calculated within the microsatellite study of *Polytrichum commune* and *Polytrichum formosum* populations, was found to be 0.8 and 0.4 respectively. Overall, high genetic diversity suggests more genetic variations in bryophytes, hence somewhat contrasting with the earlier theories of low genetic diversity in bryophyte, based on the haploid dominant life phase (Ennos, 1990; Stenoien and Sastad, 2001).

Biogeography of bryophytes

Several studies on bryophyte species showed that microsatellite loci can also be used to find the origin and evolution of species and can also help to explain the evolutionary importance of interspecific hybridization and allodiploidization in bryophyte speciation (Sastad *et al.,* 2001; Vander-Velde and Bijlsma, 2004; Sastad, 2005; Ricca and Shaw 2009; Shaw, 2009; Stenoien *et al*., 2011).

Based on microsatellite variation pattern, Stenoien *et al.* (2011) documented that *Sphagnum troendelagicum* originated before the last glacial maximum, and subsequently immigrated to central Norway by means of spores. Also, the phylogeography of five *Polytrichum* species within Europe and the presence of asymmetric reproductive isolation between the closely related taxa, *Polytrichum commune* and *Polytrichum uliginosum* (Vender-Velde and Biljsma, 2002; Vender-Velde and Biljsma, 2004) was demonstrated using microsatellite markers.

Ricca *et al*. (2008) showed that microsatellite patterns of heterozygosity are interrelated with genome size, and thus can be used to infer ploidal levels. Karlin *et al*. (2009), on the basis of microsatellite pattern, concluded that two Southern hemisphere *Sphagnum* species have triploid gametophytes. Also, Ricca and Shaw (2010) used 12 microsatellite loci and two plastid DNA markers to show allopolyploidy and homoploidy hydridization in the *Sphagnum subsecundum* complex. Further study using microsatellite markers on *Sphagnum subsecundum* complex showed the presence of asymmetric interploidal hybridization and the presence of introgression between allodiploid and haploid populations (Ricca *et al*., 2011). Even more, Karlin *et al*. (2010) reported that *Sphagnum centrale* and *Sphagnum henryense* are allopolyploids. Szovenyi *et al*. (2008) analyses indicated that both ongoing migration and ancestral polymorphism are important in explaining the intercontinental genetic similarity of peat moss populations, but their relative contribution varies with species. Microsatellites further showed that *Sphagnum cuspidatum* is one of the parental species of the double allopolyploid *Sphagnum falcatulum,* a Holantarctic species, reported in Tasmania, New Zealand and Chile. This species was found to occur on every continent except Antarctica (Karlin *et al*., 2011b).

Conclusions

The microsatellites have been utilized in several bryological researches, enabling a better understanding of this group of earliest known plants. Microsatellites are emerging as very valuable tools to study the genetic diversity, population genetics, reconstructing phylogenetic relationships among and within species, population ecology, biogeography, paternity and ploidy of bryophytes. Designing of SSR primers requires DNA sequence information, whereas the genome sequences of several bryophytes are available in public databases. Although specific difficulties, SSR markers can be developed to be used for competent studies of closely related species, through cross species amplification. As the genome sequences of more and more species of bryophytes are becoming available through EST or whole genome sequencing, the number of SSR markers is also increasing. Further advancements in microsatellite development protocols, PCR technology and computer software will facilitate the development of molecular markers that are to be used in the several areas of bryological research. Hence, microsatellites have proved to be of immense value in genetic studies of bryophytes and will also lead to novel insights into various studies of bryophytes such as reproductive biology, ecology, phylogeny and taxonomy.

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