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Natural variations in telomere lengths in different Bryophytes

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Motivation and Aim: Telomeres are specialized nucleoprotein structures at the ends of linear chromosomes in most of eukaryotes. Telomeres play an important role in protecting of the chromosomes termini from exonucleases and cell repair systems, as well as from chromosomal aberrations. In this case, the tandem telomere repeats acts as a "buffer zone" to prevent the loss of coding DNA. Telomeric DNA is shortened by the every cell division that leads to the irresistible approaching of the critical telomere length for the telomere protein complex formation and their functioning. Therefore, the telomere length is the main limitation and determination factor of chromosome protection and cell fate. On the other hand, the length of telomeres is a species-specific feature that varies in populations and individual organisms, as well as in various tissues and even on different arms of chromosomes. Plants are actually highly promising group of organisms for molecular genetics studies of evolutionary conservative mechanisms due to their sessile lifestyle and arising under it extremely flexible genetic regulation. The telomeric DNA of most plants consists of a palindrome motif $(TTTAGGG)_n$ that differs from the human telomeric repeat only for one nucleotide. In that case, bryophytes (mosses, liverworts, and hornworts) are one of the perspective groups of plants for telomere regulation studies also due to their phylogenetic position. Their study provides a unique opportunity to trace the evolution of genes associated with telomeres from the first primitive terrestrial plants to the most developed angiosperms. In addition, the slow evolution of bryophytes' genomes and its less redundancy, the predominance of the haploid gametophyte over the diploid sporophyte in lifecycles, and also simple handling in laboratory conditions, makes bryophytes one of the best model plant organisms.
The aim of the current work was to determine and characterize the natural variations of telomere lengths in different bryophyte species and ecotypes.
Methods and Algorithms: In the study we used axenic cultures of mosses: *Physcomitrium patens*, 4 ecotypes (Gransden, Great Britain; Reute, Germany; Villersexel, France; Kaskaskia, USA); *Ceratodon purpureus* (male R40 and female GG1 lines), and natural isolates of peatmoss *Sphagnum fallax* MW (USA), *Sphagnum girgensohnii* (Russia, Sverdlovsk region) and *Sphagnum sp*. (Russia, Republic of Mari-El). Mosses were grown on a solid mineral medium BCD with the addition of ammonium tartrate. To analyze the telomere length, genomic DNA isolated from 14-day-old protonema of *P. patens* and *C. purpureus* or *Sphagnum* gametophores were used. Telomere length analysis was performed by TRF (Terminal Restriction Fragment analysis) coupled with Southern blot analysis. The average telomere length was calculated using the TeloTool software.
Results: We have shown that different ecotypes of *P. patens* plants have a telomere length in the range from 1000 to 1500 bp. It was 1.5-3 times shorter than the telomere

length of the model angiosperm plant *A. thaliana* Col-0 (2500-4500 bp in the average). The average telomere length in the Gransden, Reute and Villersexel ecotypes was ~ 1300 bp. However, the average telomere length in the Kaskaskia ecotype was slightly shorter (~ 1200 bp). In addition, we have shown that all the ecotypes studied have specific telomeric sequences, presumably of intra-chromosomal localization, and differing in their location and length within the ecotypes.

It was shown that the telomere length of the female plant *C. purpureus* GG1 ranges from 480 bp to 500 bp, while in the male plant *C. purpureus* R40 ranges from 900 bp to 1000 bp. Thus, the telomere length in male and female plants significantly differs for about two times, which, presumably, may be due to the genetic factors associated with U or V sex chromosomes. In addition, we have shown that only male line of *C. purpureus* R40 had abundant telomeric DNA repeats out of the main telomere length signal on chromosomes, which may be intrachromosomal telomeric DNA. Also the dynamics of telomere length maintaining was studied both for *C. purpureus* GG1 and *C. purpureus* R40 protonema (56 and 42 days respectively). It was shown, that the telomere length did not change in both the female line GG1 and the male line R40, which indicates the stability of the telomeric complex and the system of regulation of the length of *C. purpureus* telomeres during active cell division at the protonema growth stage.

The telomere lengths of *S. fallax* MW and *S. girgensohnii* were about 1880–2000 bp. However, telomeres were slightly shorter in another isolate of *Sphagnum sp* (the Mari-El isolate), whose telomere length ranged from 1100 to 1500 bp. Identification of intra-chromosomal telomeric tandem DNA repeats was carried out by exonuclease restriction using the enzyme Bal31. A strong signal of intrachromosomal telomeric repeats was identified in *S. fallax* MW DNA (~ 2000 bp). However, such interstitial telomeric DNA has not been detected in *Sphagnum sp*. and *S. girgensohnii*. Therefore, it was shown that there are intraspecies variations in telomere length of *Sphagnum* and also differences in the telomeric repeats location on chromosomes.

Conclusion: Therefore, we have shown that the telomeric DNA of bryophytes is shorter than the telomeric DNA of angiosperms. The telomere length of bryophytes can vary both between species and within one species. The telomere length in *P. patens* did not vary within the species. The telomere length of *C. purpureus* differs between male and female plants. The telomere length of *C. purpureus* GG1 and R40 lines did not change during the life cycle at the protonema stage. Telomere length in various *Sphagnum* species ranged from 1100 to 2000 bp. However, we found differences in the localization of telomeric DNA repeats in *Sphagnum* species. Further study of the biology of bryophyte telomeres will provide new data on the mechanisms of regulation of telomere length and their functional activity in terrestrial plants.

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