Analysis of chromosome termini in potato varieties

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ABSTRACT

Chromosomes of potato (*Solanum tuberosum*) are terminated by telomeres, which are formed by tandemly repeated [TTTAGGG]n oligonucleotide sequence. The total length of blocks of telomeric DNA has been known to vary largely among plant species and their varieties, and also among individual chromosome arms within a single nucleus. To check for such differences in potato varieties, which could be of a possible use in genotyping, we performed pulsed-field gel electrophoretic analysis of terminal restriction fragments in selected potato varieties. We found a typical range of telomere lengths of 20–60 kb in most analysed varieties. In one of analysed varieties (Monalisa), telomeres of wider span (up to 80 kb) have been observed. Most of restriction enzymes (*Pvu*II, *Hae*III, *Taq*I) produced a resulting smeared hybridisation pattern of telomeres. When using *Bgl*II, however, a doublet hybridisation band could be observed. This may reflect differences in composition of telomere-associated sequences at different chromosome ends.

Keywords: plant chromosomes; telomere length; potato varieties; terminal restriction fragments

Telomeres are nucleoprotein structures forming and protecting ends of eukaryotic chromosomes. Their DNA part usually consists of tandemly repeated simple oligonucleotide sequences synthesised by a specific ribonucleoprotein enzyme complex of reverse transcriptase activity – telomerase. The first characterized plant telomeric sequences were cloned from *Arabidopsis thaliana* (Richards and Ausubel 1988) and it was shown that these are composed primarily of tandemly repeated blocks of the sequence 5'-CCCTAAA-3' and are heterogeneous in length (2–5 kb). Homologous telomeric sequences were observed in *Zea mays* (corn) and the same telomeric sequence, but forming considerably larger arrays (30–60 kb) was then found in *Solanum esculentum* (tomato) (Ganal et al. 1991) and later in a number of other plant species including *Hordeum vulgare* (barley) (Schwarzacher and Heslop-Harrison 1991, Roder et al. 1993), *Oryza sativa* (rice) (Wu and Tanksley 1993a, b) and *Nicotiana tabacum* (tobacco) (Suzuki et al. 1994, Fajkus et al. 1995). In addition to a wide variation of telomere lengths between species and between varieties of one species, lengths can vary also within a single species; analysis of 22 inbred lines of maize showed that they varied more than 25-fold (Burr et al. 1992).

In this study, we report on determination of telomere lengths in a set of *Solanum tuberosum* (potato) varieties to gain knowledge of a general size range among telomeres within a given genome and its possible variation between varieties.

MATERIAL AND METHODS

Leaf blades of adult plants of *Solanum tuberosum* L. cv. Granola, Keřkovské rohlíčky, Satina, Désirée, Karin, Kobra and Monalisa were used to prepare high-molecular-weight DNA preparations for pulsed-field gel electrophoresis. To determine the lengths of terminal restriction fragments, TRF (reflecting the size of the telomeric TTTAGGG repeat), DNA samples embedded in agarose blocks (3–5 µg) were equilibrated with restriction buffer and digested with 40 U of appropriate restriction endonuclease (as indicated in Figure 1) in a fresh portion of buffer for 16 h. Electrophoresis was performed on the Gene Navigator System (Pharmacia Biotech, Sweden) using 1% Fast Lane (FMC) agarose gel in 45 mM Tris-borate, 1 mM EDTA, pH 8.0. The running conditions (pulse ramping time from 5 s to 50 s, voltage 180 V, temperature 10°C, time 22 h) enabled separation of fragments between 50–800 kb in size. After electrophoresis, gels were stained by ethidium bromide, photographed and alkali-blotted onto a Hybond N+ membrane. TRF were detected by hybridisation with an end-labelled $(CCCTAAA)_6$ probe and visualised on X-ray film or on a Phosphoimager STORM (Molecular Dynamics).

RESULTS AND DISCUSSION

Analysis of telomere lengths in selected potato varieties revealed a size range of 15–60 kb (Figure 1) being similar throughout the varieties tested with the only exception of Monalisa, where the upper limit of telomere size was shifted to 80 kb. The size range of potato telomeres thus corresponds well with the previously determined TRF size range in tomato (30–60 kb, Ganal et al. 1991) and is shorter than that of tobacco (20–160kb, Kovařík et al. 1996), the two most precisely mapped relatives of potato from *Solanaceae* family.

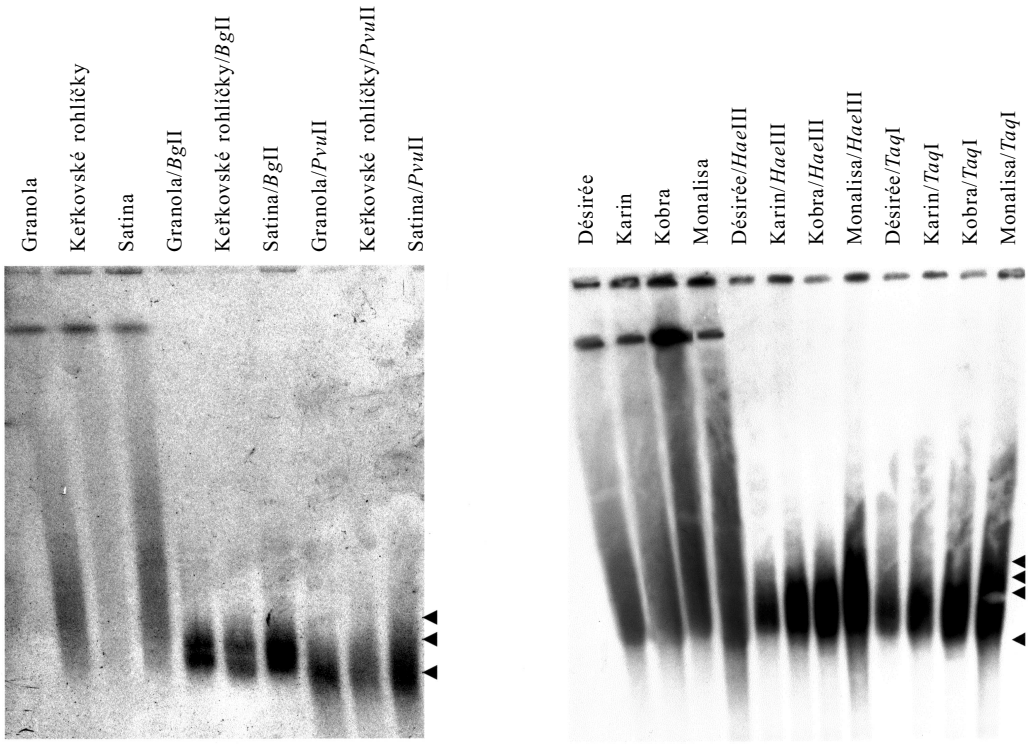

Figure 1. Southern hybridisation of high-molecular-weight DNA of selected potato varieties with radioactively labelled telomeric probe $[TTTAGGG]_4$ after separation by pulsed-field gel electrophoresis; variety names and restriction enzymes used are given above each lane, positions of marker bands (panel A – MidRangeII Marker, NEB, panel B – MidRangeI Marker, NEB) are shown by arrowheads; control lanes with no restriction cleavage are shown on the left of each panel

A smeared pattern of TRFs was obtained in most restriction enzymes used. Although the appearance of TRF is affected by the choice of the restriction enzyme due to a different frequency of target sites in subtelomeric regions, no significant differences in the telomere size range can be observed when comparing enzymes with four- and six-nucleotide recognition sequence. TRF patterns using restriction enzyme *Bgl*II show two distinct bands of telomere-specific signal corresponding to sizes of 30 and 50 kb, respectively, in cv. Granola, Keřkovské rohlíčky and Satina (Figure 1A). Thus, using this restriction enzyme, it is possible to distinguish two size-classes of TRF in potato genome which are probably defined by the presence or absence of a long array of subtelomeric tandemly repeated DNA sequence lacking *Bgl*II restriction site. Consequently, the distance of the first *Bgl*II site from the telomere-subtelomere junction varies at different chromosome ends with the occurrence and length of this repeat array. The alternative explanation by two length fractions of telomeres themselves is excluded by results obtained on the same varieties with a different enzyme, *Pvu*II (Figure 1A).

We conclude that potato, as well as all the other species from *Solanum* and *Nicotiana* genera tested so far belongs to a group of long-telomere plants (Fajkus et al. 1998). Variation in the average telomere size between tested varieties is relatively low, but our results suggest differences in arrangement of DNA sequences forming junctions between telomere and subtelomere at individual chromosome ends.

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ABSTRAKT

Analýza konců chromozomů u vybraných odrůd bramboru

Chromozomy bramboru (*Solanum tuberosum*) jsou ukončeny telomerami, které jsou tvořeny tandemově opakovanou [TTTAGGG]n oligonukleotidovou sekvencí. Je známo, že celková délka bloků telomerické DNA se velmi liší mezi jednotlivými rostlinnými druhy a jejich odrůdami a také mezi jednotlivými chromozomálními raménky v rámci téhož jádra. Pro zjištění takových rozdílů u vybraných odrůd bramboru, které by bylo možno využít k identifikaci odrůd, jsme použili analýzu koncových restrikčních fragmentů pomocí pulzní gelové elektroforézy (PFGE). U většiny odrůd byly zjištěny délky telomer v typickém rozmezí 20–60 kb. U jedné analyzované odrůdy (Monalisa) byly pozorovány telomery o širším délkovém rozpětí (do 80 kb). Většina restrikčních enzymů (*Pvu*II, *Hae*III, *Taq*I) poskytovala obraz telomer v podobě rozmazaného hybridizační pásu. Při použití *Bgl*II však byla pozorována dvojice hybridizačních pásů, což může odrážet rozdíly ve složení telomer-asociovaných sekvencí na jednotlivých koncích chromozomů.

Klíčová slova: rostlinné chromozomy; délka telomer; odrůdy bramboru; terminální restrikční fragmenty

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