

Structural Variability of Subtelomeres Applied for Genotyping of Selected Potato Varieties

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Abstract: Subtelomeric regions reveal a relatively high degree of polymorphism due to the increased frequency of recombination events in these chromosome loci. In a search for molecular markers applicable to genotyping of potato varieties, we focused on two possible sources of polymorphism occurring in this region: (i) arrangement of blocks of subtelomeric chromatin; (ii) structure of telomere-subtelomere boundary. Analysis of the internal arrangement of subtelomeric sequences showed several types of cultivar-specific spectra of PCR products arising from the variant orientation of sequence units of the ST3-subtelomeric sequence, or from different lengths of regions linking the individual sequence units. Further, the telomere-subtelomere boundary sequences were amplified using telomeric and ST3-specific primers and the obtained products were cloned. Sequence analysis of the clones resulted in characterisation of a novel telomere-associated sequence (FIN2). Primers derived from this sequence were then used alone or in combination with telomeric or ST3-specific primers to generate cultivar-specific spectra of PCR products. The described combinations of sequence-specific primers may be used for the fast, cheap and reproducible PCR-genotyping of selected potato varieties.

Keywords: genotyping; potato; PCR; subtelomere; telomere-associated DNA sequences

Techniques for a reliable identification of cultivars of agriculturally important crops are requested by breeders and farmers. This also concerns the potato (*Solanum tuberosum* L.), which belongs to the most important agricultural products and which was produced in 116 varieties in the Czech Republic in 2003. Several kinds of markers have been developed for potato molecular genotyping, namely RAPD (Random Amplified Polymorphic DNA) and SSR (Simple Sequence Repeat DNA polymorphism) markers. Although the techniques have been successfully applied (DONG *et al.* 2005;

SOBOTKA *et al.* 2004), they share a common problem of relatively low reproducibility when comparing results between different laboratories, namely in the case of RAPD technique. Therefore, we aimed to develop a set of sequence-specific markers providing a reproducible discrimination of selected potato varieties. As a source of DNA sequences which would be specific to potato genome while showing a high degree of natural polymorphism, sequences derived from subterminal loci of potato chromosomes were selected. The reason for choosing subtelomeres is their frequent involvement

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in recombination events which result in a high occurrence of rearrangements in these chromosome loci (MIKHAILOVA *et al.* 2001). Although subtelomeres of a given species are usually formed by a limited number of repeated DNA sequence families, their mutual arrangement, attachment to telomeres and the presence of interspersed unique or low-copy-number sequences make these terminal domains chromosome specific (SÝKOROVÁ *et al.* 2003a). Similarly, the frequent recombination events may give a rise to such rearrangements among plant cultivars.

To establish the PCR-based genotyping strategy for potato cultivars, we utilised the information on the components of potato chromosomes. We have shown recently that potato telomeres are composed of long arrays (20–60 kb in most cultivars) of tandemly repeated [TTTAGGG]_n sequence (FAJKUS *et al.* 2002). Interestingly, this kind of telomeres is not common to all *Solanaceae* genera. Our recent results show that *Vestia*, *Sessea* and *Cestrum* genera lack this “typical” type of plant telomeres (SÝKOROVÁ *et al.* 2003b, c). As a prototype of potato subtelomeric sequence, the previously characterised ST3 sequence (GenBank X63738) was chosen (ROKKA *et al.* 1998). Having set the telomeric and subtelomeric starting posts, the arrangement of ST3 sequence units and the boundary between ST3 and telomere could be analysed. Here we report the results of this analysis and describe a novel telomere-associated sequence FIN2 (GenBank AY631046). We further show that combinations of PCR primers derived from telomeric, ST3 and FIN2 sequences make it possible to determine selected potato cultivars.

MATERIAL AND METHODS

Plant material. Leaf blades of adult plants of *Solanum tuberosum* L. cv. Apolena, Asterix, Beladonna, Dali, Désirée, Granola, Impala, Karin, Keřkovské rohlíčky, Kobra, Komtesa, Kornelie, Monalisa, Ornella, Pacov, Provento, Rosara, Satina, Vladan and Zlata were used to isolate DNA (DELLAPORTA *et al.* 1983).

Oligonucleotide primers and PCR conditions. To obtain the basic sequence unit of the ST3 sequence, the following primers, heading inside the ST3 monomer, were designed (Figure 1): STFORIN 5'-ACCTCCTTCAATTGGTATGA-3'; STREVIN 5'-GAGCCCCTAAGATGTTCTAT-3'.

PCR was performed using 200 ng of template genomic DNA under the following conditions:

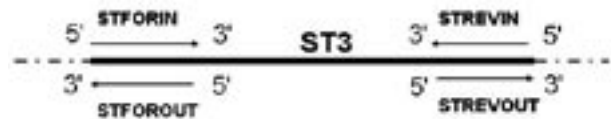


Figure 1. Schematic arrangement of ST3-specific primers on the ST3 sequence (GenBank X63738)

initial denaturation (95°C/3 min), 35 cycles (95°C per 45 s, 58°C/45 s and 72°C/90 s) and final extension 72°C/6 min.

For the analysis of arrangement of ST3 sequence units, additional primers were designed heading outside the ST3 monomer unit (Figure 1) to amplify either spacer sequences between the ST3 sequences or the tandemly arranged copies of the ST3 sequence ST: STFOROUT 5'-TCATACCAATTGAAGGAGGT-3'; STREVOOUT 5'-ATAGAACATCTTAGGGGCTC-3'. To avoid a possible elimination of longer PCR products, the Expand High Fidelity System (Roche) was used with 200 ng of template DNA under the following conditions: initial denaturation (94°C/2 min), 10 cycles (94°C/15 s, 53°C/30 s and 68°C/4 min) followed by 20 cycles with the same parameters but with a 20 s increment of extension time in each cycle. Final extension was performed at 72°C for 6 min.

For isolation and characterisation of telomere-subtelomere junctions, each of the ST3-specific primers was used in combination with the telomere C-strand primer TELPR: 5'-CCGAATTC AAC-CCTAACCTAAACCCTAAACCC-3'. The Expand High Fidelity system was used with 100 ng of template DNA (cv. Satina). After initial denaturation (94°C/2 min), 10 cycles of amplification (94°C/30 s, 55°C/30 s and 68°C/6 min) were performed using only the ST3-specific primer. Then the TELPR primer was added and PCR proceeded for 35 cycles (94°C/30 s, 55°C/30 s and 68°C/4 min) with a 20 s increment of extension time per cycle.

Agarose gel electrophoresis. PCR products were separated in 1% agarose or (where stated in figure legends) in 2% NuSieve Agarose gels (Cambrex), in 1 × TBE (90mM Tris-borate, 2mM EDTA) at 2–3 V/cm. The gels were stained with ethidium-bromide (0.5 µg/ml) and the DNA was detected in UV-light using DIANAIII documentation system (Raytest).

Cloning and sequencing. PCR products from telomere-subtelomere junctions were ligated into pZErO/Kan vector (Invitrogen) linearised with

EcoRV restriction endonuclease. Recombinant clones were sequenced using an AmpliCycle sequencing kit (Applied Biosystems) and ABI PRISM 310 sequencer (Perkin-Elmer).

RESULTS

Occurrence of the ST3 sequence in potato cultivars

PCR screening of selected potato cultivars using STFORIN and STREVIN primers shows ubiquitous presence in the tested potato cultivars. It can be

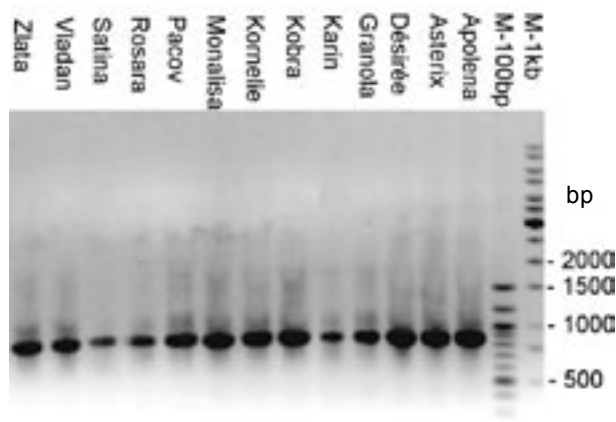


Figure 2. Screening of potato cultivars for the presence of ST3-monomer unit. PCR was performed using STFORIN and STREVIN primers. All tested cultivars show the product of the expected ST3-unit length (845 bp). M-100bp and M-1kb lanes contain 100 bp DNA ladder (NEB) and GeneRuler™ 1 kb DNA ladder (MBI Fermentas) markers, respectively

seen in Figure 2 that all tested potato cultivars revealed the presence of the PCR products of the same length (845 bp) corresponding to the size of the monomer sequence unit.

Analysis of mutual arrangement of the ST3-family members

Using the STRFOROUT and STREVDOUT primers heading outside the ST3 sequence unit differential patterns of PCR products were obtained. 23 out of 37 cultivars showed the presence of about 5 kb-long PCR product (Figure 3) suggesting the most frequent genomic spacing of the ST3 sequence. Multiple variants of shorter spacing (1.2–3.8 kbp) were observed in 18 cultivars, either alone or in combination with the 5 kb-long product. Three cultivars (Apolena, Dali, Provento) showed the absence of any PCR product, thus suggesting the absence of closely spaced ST3 units in these genomes. Altogether, 12 different types of electrophoretic patterns could be distinguished using these two primers. The regular ladder which would suggest a tandem arrangement of the ST3 sequence units was not observed in any cultivar.

Analysis of telomere-subtelomere junction

Since another source of polymorphism is expected to result from the variant attachment of ST3 sequence to telomere, the junction fragments were amplified and cloned as described in the Material and Methods section. Besides a number of clones containing only degenerated telomeric repeats (typical of telomere-associated chromosome

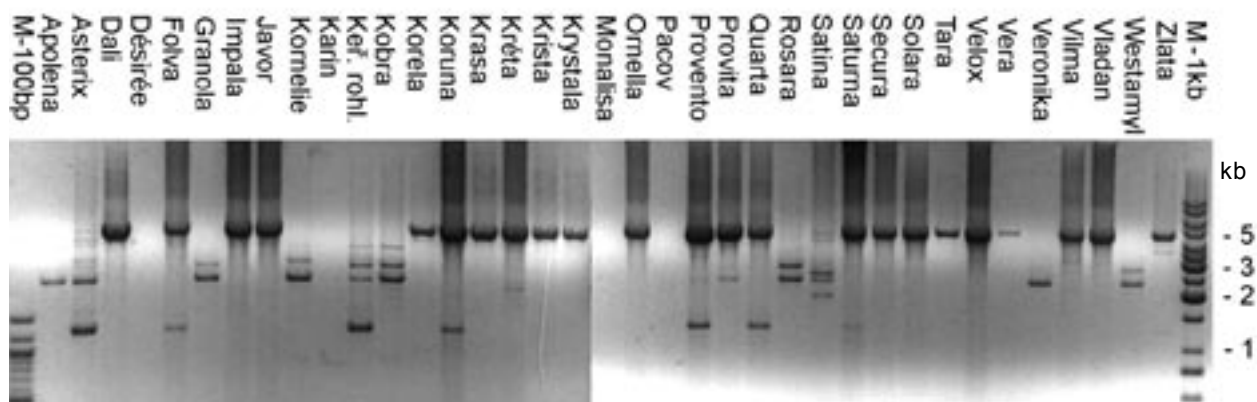


Figure 3. Analysis of mutual arrangement of the ST3-units in potato cultivars using the STRFOROUT and STREVDOUT primers. M-100bp and M-1kb lanes contain 100 bp DNA ladder (NEB) and GeneRuler™ 1 kb DNA ladder (MBI Fermentas) markers, respectively

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1  accctcattca atgggtatga agccaattca gctttctctc tggagatcc tcccatogca
   STFORIN →
61  tcccaaatit tgcgaatctc atccaogaat ccttggggat ctctcctaat tctagagcct
121  aaaaagatag gaggattcat cctcaogoaq tctctcaacc ttggggctac cgtactctca
181  taagcattca ccttaggcc cctatctctc tttgcttgag ccgtcotggc ttgggctagg
241  gtcacaaaag ccgctctaat ctattcatta gtcctggccg ggggaaaccac cgggacctca
301  ttctcttget cccctatagg aacttgATTA CATTACATAc CTTGACCATT TTCCACAAct
361  TGAGGACGAA CTCCCTCAtC ACCTAACTCA TCTTCTCTC TCCTTcGATT CGTCCTCGTA
   FIN2UP →
421  TTCATTTCTT ATAAACACAA GAGAAGGATT AGGAAAATGA CACTTATAAA CTTGCCCAA
481  AACTCACTGA TTTCCACAC TTTATCAAAT TTCCAGGTTc AGAACCTTTT TTCCAAAAGT
541  GACTACTTCC AATTTTATAA CTCCAATTTA TGCITAAACT TTAaaaaATC ATACGTTAGG
   ← FIN2DO
601  CTCTAGTTTC ACCTATCTAT Ttttagggtc gctacaaggc cttagagttt aggaataata
681  attttgggtt tagggtttag gatatagggt ttagggttag gatttaggtt ttagggttta
721  gggttttgag tttagggttt agggttttag gtttagggtt gaattc
   ← TELPR

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Figure 4. Nucleotide sequence of the insert of pFIN2 clone (GenBank AY631046). Positions of primers STFORIN, FIN2UP, FIN2DO and TELPR are underlined and their 5'-3' orientations are marked with arrows. The part which is homologous to pST3 sequence (GenBank X63738) is given in plain lower case letters, and the region showing homology to *Solanum tuberosum* resistance gene cluster (GenBank AF265664) is in italics. Degenerated telomeric repeats are given in lower case bold letters

regions), one of the clones termed as pFIN2 (coming from the PCR reaction with STFORIN and TELPR primers) contained a 766 bp insert (Figure 4). Sequence alignment to the known database sequences revealed that the 5'-end terminus is formed by an incomplete unit of the ST3 sequence (position 1–320) followed by a sequence (FIN2) which was directly attached to degenerated telomeric repeats. Within this sequence, a region (468–556) homologous to the GenBank sequence AF265664 (*Solanum tuberosum* resistance gene cluster) was found. The complete sequence was submitted to GenBank and assigned accession number AY631046.

For further analysis, primers were designed to amplify the FIN2 region (outside the ST3 sequence and telomeric repeats). These were termed as FIN2DO (5'-GAAACTAGAGCCTAACGTATGAAT-3') and FIN2UP (5'-GAACTCCCTCATCACCTAACTCA-3'). These primers amplify a 243 bp region in the FIN2 clone. When applied to the set of tested potato cultivars, differential patterns of PCR products are generated (Figure 5). These primers can also be used in combination with the above described

ST3-specific primers or with the telomeric primer TELPR (Figures 6 and 7), thus extending possibilities of reliable genotyping of cultivars.

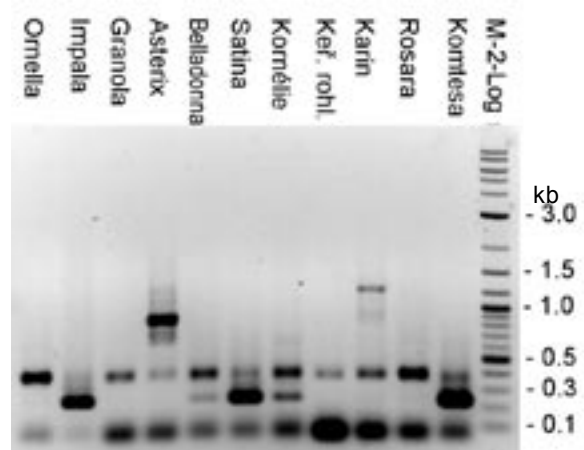


Figure 5. Differential pattern of PCR products obtained using FIN2DO and FIN2UP primers (specific to telomere-associated sequence FIN2). Marker lane (M-2-Log) contains 2-Log DNA ladder (NEB)

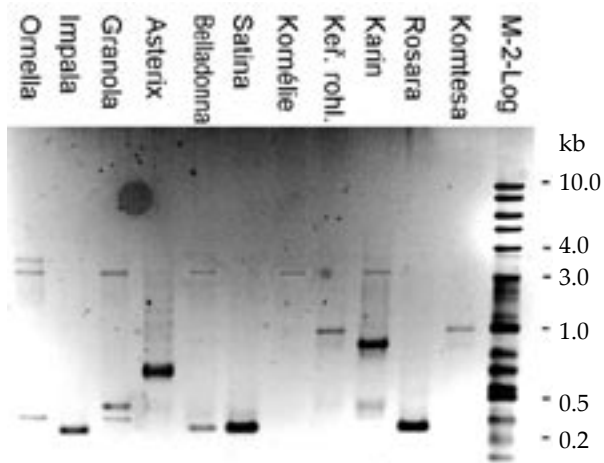


Figure 6. Electrophoretic pattern of PCR products obtained using STFORIN and FIN2DO primers in potato cultivars. Marker lane (M-2-Log) contains 2-Log DNA ladder (NEB)

DISCUSSION

In this paper we described a PCR-based assay utilising variability of sequence arrangement in subterminal chromosome regions of potato cultivars. To establish this assay, primers were designed either according to the previously described potato telomeric and subtelomeric sequences or based on the newly described telomere-associated potato sequence. Besides the directly applicable genotyping method using an appropriate combination of the above-mentioned oligonucleotide primers, this study also revealed some aspects of structural organisation of potato genome. E.g. the previously described ST3 subtelomeric sequence does not form any simple tandem repeats, but it rather occurs within a genome as a part of a larger complex repeat. This can be deduced from the generation of about 5 kb-long PCR products in most tested cultivars when using primers STFOROUT and STREVOUT. The telomere-associated sequence obtained from the FIN2 clone (derived from cv. Satina) shows ubiquitous presence among the tested cultivars (similarly like the ST3 sequence), but the region flanked by FIN2UP and FIN2DO primers varies in length among cultivars (Figure 5). We conclude that the described polymorphisms or their combinations can be easily exploited for genotyping of potato cultivars using 2–3 independent PCR reactions. Moreover, the described sequence-specific primers which are designed on the basis of sequencing data can be used under

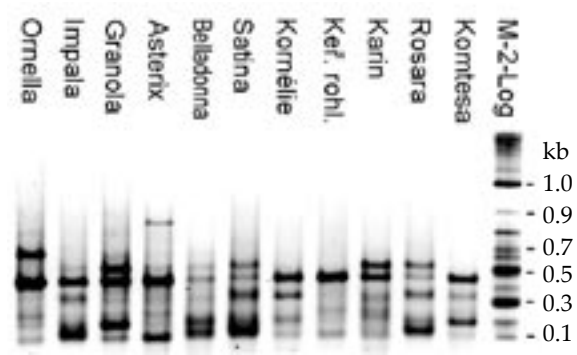


Figure 7. Electrophoretic pattern of PCR products obtained using FIN2UP and TELPR primers in potato cultivars. In contrast to the other experiments shown, electrophoresis was performed in 2% NuSieve Agarose (Cambrex). Marker lane (M-2-Log) contains 2-Log DNA ladder (NEB)

high-stringency annealing conditions providing thus results of higher reproducibility than short arbitrary primers. Therefore, the described primers may provide a useful alternative to the commonly used RAPD markers (PRZETAKIEWICZ *et al.* 2002).

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Souhrn

NEPLECHOVÁ K., SÝKOROVÁ E., NOVOTNÁ M., PTÁČEK J., FAJKUS J. (2005): **Strukturní variabilita subtelomer použitá pro genotypizaci vybraných odrůd bramboru**. *Czech J. Genet. Plant Breed.*, **41**: 17–22.

Subtelomerické oblasti se vyznačují relativně vysokým stupněm polymorfismu vzhledem ke zvýšené frekvenci rekombinací v těchto chromozomálních lokusech. Při hledání molekulárních markerů použitelných pro genotypizaci odrůd bramboru jsme se zaměřili na dva možné zdroje polymorfismů, vyskytující se v této oblasti: 1. uspořádání bloků subtelomerového chromatinu; 2. struktura rozhraní telomera-subtelomera. Analýza vnitřního uspořádání subtelomerových sekvencí ukázala několik typů odrůdově-specifických spekter produktů PCR, která vznikají v důsledku variantní vzájemné polohy základních jednotek subtelomerové sekvence ST3 nebo rozdílných délek spojovacích sekvencí mezi nimi. S použitím telomerového primeru v kombinaci s některým ze ST3-specifických primerů byly dále amplifikovány a následně klonovány hraniční úseky mezi telomerou a subtelomerou. Výsledkem sekvenční analýzy získaných klonů byla charakterizace nové telomer-asociované sekvence (FIN2). Primery odvozené z této sekvence pak byly použity samy nebo v kombinaci s telomerovým nebo některým ST3-specifickým primerem k získání odrůdově-specifického spektra PCR produktů. Popsané kombinace sekvenčně-specifických primerů mohou být použity pro rychlou, levnou a reprodukovatelnou genotypizaci vybraných odrůd bramboru.

Klíčová slova: genotypizace; brambor; PCR; subtelomera; telomer-asociované DNA sekvence

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