

SSR and retrotransposon based markers and their application to potato varieties identification



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INTRODUCTION

The unambiguous identification of agricultural and horticultural crop varieties is important in many areas of agriculture during their breeding and registration process, seed-production, trade, inspection and also for plant biology research. New techniques based on DNA profiling provide novel approaches to variety identification which offer advantages over traditional morphological comparisons.

The advantages of techniques based on PCR include great potential resolving power, testing at all stages of development, cost effectiveness, and also data that can be analyzed objectively. Molecular markers can be than a modern and suitable tool for variety identification.

The Czech list of Registered Potato Varieties includes hundred and seventy-eight potato varieties in 2007. In this study we analyzed set of accessible potato varieties (*Solanum tuberosum* L.) by several methods.

The goal of this study was to design a set of molecular markers for identification of potato varieties cultivated in the Czech Republic. Final set of methods and molecular markers can be used by certification and testing agencies in Czech Republic, for example by Czech Agriculture and Food Inspection Authority (SZPI). Results will be used for enrichment of electronic filing "Catalogue of Registered Potato Varieties".

RESULTS

We obtained the set of fingerprints for available 152 potato varieties and we try to fill our database.

Example of electronic catalogue of registered potato varieties

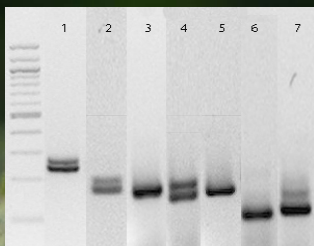
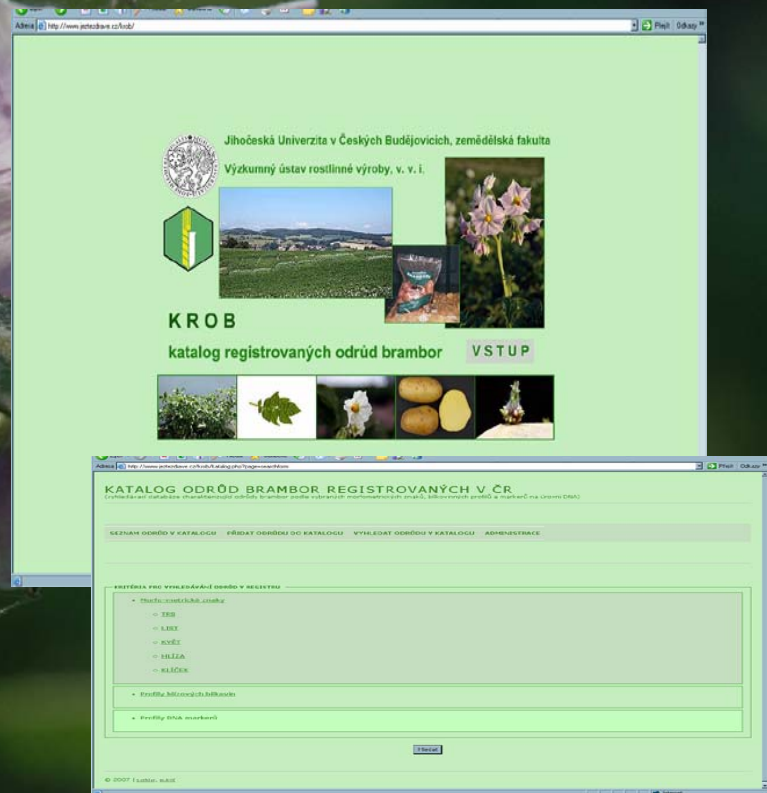


Fig.1. Example of electrophoreogram-3% agarose-synergel. SSR analyses: potato variety ADORA 1 - primers STS1+2, (m.w. 260,230), 2 - primers STWIN 12G (m.w. 200,180), 3 - primers STM 1102 (m.w. 170), 4 - primers STM 2005 (m.w. 180,160), 5 - primers STM 3012 (m.w. 160), 6 - primers STM 3015 (m.w. 110) and 7 - primers STG BBS (m.w. 180,160), 15 DNA ladder marker 100bp.

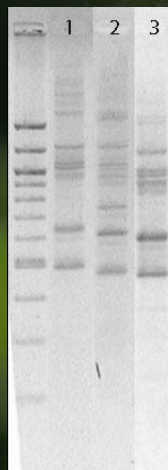


Fig.2. Example of electrophoreogram-3% agarose-gel. Retrotransposon analyses: potato variety ADORA 1 - primer P-Tst-1 (m.w. 1240, 1060, 1040, 970, 640, 400), 2 - primer P-Tst-3 (m.w. 1240, 1070, 1030, 970, 800, 760, 700, 680, 630, 380), 3 - primer P-Tst-6, (m.w. 1190, 1020, 980, 910, 850, 600, 570, 370), 15 DNA ladder marker 100bp.

CONCLUSION

We obtained the set of 14 markers (10 based on microsatellites and 4 based on retrotransposon). Analyses still continue as well as the filling of database.

We found that all tested methods are applicable for variety identification, but for identification of wide range of varieties it seems to be more suitable to use the complex set of molecular and morphological markers.

MATERIAL AND METHODS

Plant material. Set of available registered potato varieties was used for analyses respectively 152 potato varieties. Material was rendered by ÚKZÚZ Lipa u Havlíčkova Brodu.

Methods. The DNA extraction was done from tuber juice by the Invisorb Spin Plant Mini Kit (INVITEK).

PCR-MICROSATELLITES analyses.

PCR-SSR analyses were chosen primers STM 2005, STM 1102, STM 3012, STM 1106, STM 3015, STM1052, STM1097, STWIN 12G, STIIKA, STS1+2 and STG BBS.

PCR-ISSR analyses were chosen primers P1, P2, P3, P4 a B1.

PCR-RETROTRANSPON analyses. In this case were chosen primers P-Tst-1, P-Tst-3, P-Tst-6.

The analyses were done according to the standard protocol – Biotechnological centre, Agriculture faculty, University of South Bohemia

(http://www.samos.cjamos/bc/modules/low/kurz_cbsah.php?kod_kurzu=bc_154)

Abstrakt:

The unambiguous identification of agricultural and horticultural crop varieties is important in many areas of agriculture during their breeding and registration process, seed-production, trade, inspection and also for plant biology research. New techniques based on DNA profiling provide novel approaches to variety identification which offer advantages over traditional morphological comparisons. Traditional approach to variety identification is carried out by the observation and recording of a range of morphological characters or descriptors. Such an approach is precise but time-consuming and it is less suitable when results are required rapidly and only seed samples are available. The advantages of techniques based on PCR include great potential resolving power, testing at all stages of development, cost effectiveness, and also data that can be analyzed objectively. Molecular markers can be than a modern and suitable tool for variety identification. The Czech list of Registered Potato Varieties includes hundred and seventy-eight potato varieties present in 2007. In this study we analyzed set of accessible potato varieties (*Solanum tuberosum* L.) by several methods as PCR-SSR, PCR-IRAP and PCR-RBIP. The goal of this study was to design a set of molecular markers for identification of potato varieties cultivated in the Czech Republic. Resulted set of methods and molecular markers can be used by certification and testing agencies in Czech Republic, for example by Czech Agriculture and Food Inspection Authority (SZPI). Results will be used for enrichment of electronic filing "Catalogue of Registered Potato Varieties". We found that all of tested methods are applicable for variety identification, but for identification of wide range of varieties it seems to be more suitable to use the complex set of molecular and morphological markers. This study was supported by grants MSM 60076658-06, NAZV 1B44011, GACR 521/08/H042, IG05/07.