Molecular markers and their application in potato variety identification and GMO testing

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INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is along with wheat, rice and maize, one of the four most valuable world crops. The improvement and creation of new varieties with new combinations of current features or essentially new features, such as GMO potatoes or conventional varieties with better parameters of quality or resistance to biotic and abiotic factors, is one of the main goals of plant breeding.

The Czech list of Registered Potato Varieties includes hundred and seventy-eight potato varieties in 2007. Our aim is 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".

Genetically modified (GM) or transgenic crops, now more often called "Biotech crops" are commercially cultivated since 1996. The global area of approved biotech crops in 2006 was 102 million hectares. In 2006, 22 countries grew biotech crops. The Czech Republic is one of the six EU countries where biotech crops are cultivated at present. We focused on developing of fast, precise and cheap method based on PCR to detect the presence of transgenes in potatoes – tubers and leaves, which allows monitoring the presence of GM potatoes in market, environment, etc. and to quantify "contamination" of ware potatoes (tubers) with GMOes.

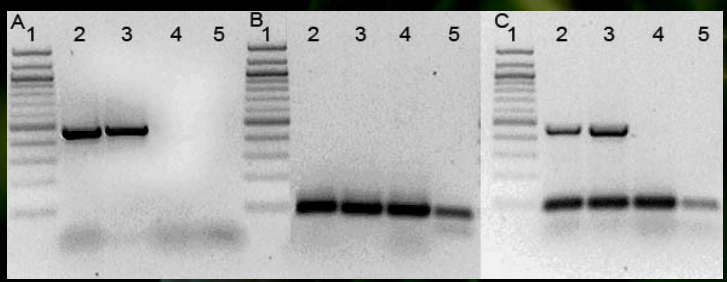


Fig.1. Example of electrophoreogram-1,5 % agarose gel. A - Uniplex PCR analyses, primers pair GNA for lectin, B - Uniplex PCR analyses, primers pair UGP for endogen, C - Multiplex PCR analyses both primers pair. Transformed Desiree DNA extracted from tuber (2) and leaf (3) and Desiree GMO free as a negative control tuber (4), leaf (5) and (1) DNA ladder marker 100bp.

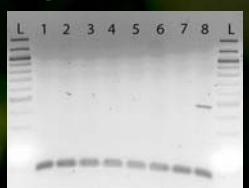
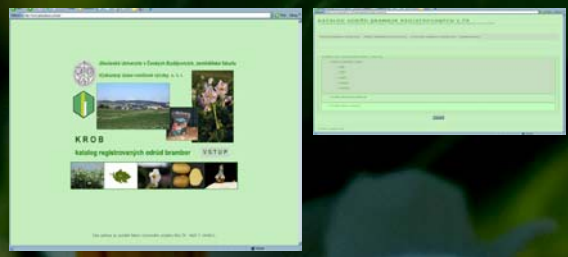


Fig.2. Example of electrophoreogram-1,5 % agarose gel. Duplex PCR analyses primers pair GNA and UGP. Transformed Desiree DNA extracted from tuber per cent of transgene: 1 - 0,1%, 2 - 0,5%, 3 - 1%, 4 - 1,5%, 5 - 2%, 6 - 3%, 7 - 4%, 8 – 5%, L - DNA ladder marker 100bp.

Example of GMO detection by duplex PCR

RESULTS



Example of preparing database or catalogue of registered potato varieties

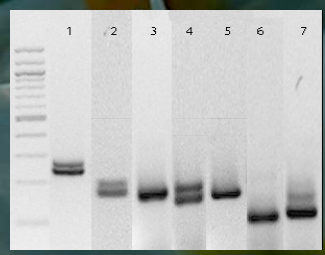


Fig.3. Example of electrophoreogram-3% agarose-synergel. SSR analyses: potato variety ADORA 1 - primers STS1+2, (m.w. 260,230), 2 - primers STWIN 12G (m.w. 200,180), 3 - primers STM 1102 (m.w. 170), 4 - primers STM 2005 (m.w. 180,160), 5 - primers STM 3012 (m.w. 160), 6 - primers STM 3015 (m.w. 110)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

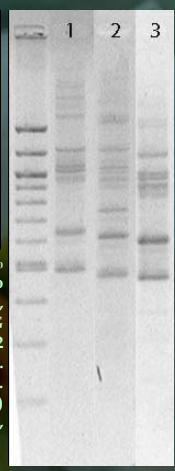
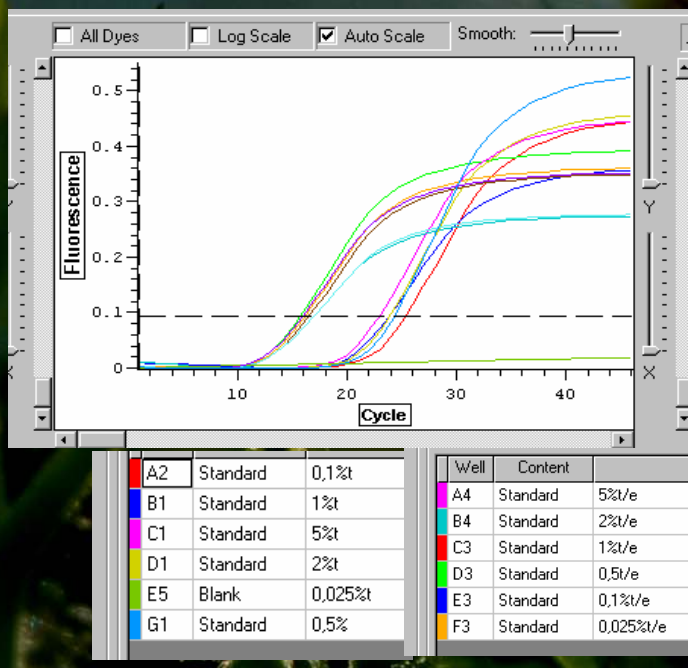


Fig.4. Example of electrophoreogram-3% agarose-gel. Retrotransposon analyses: potato variety ADORA 1 - primer P-Tst-1 (m.w. 1240, 1060, 1040, 970, 640, 400), 2 - primer P-Tst-3 (m.w. 1240, 1070, 1030, 970, 800, 760, 700, 680, 630, 380), 3 - primer P-Tst-6. (m.w. 1190, 1020, 980, 910, 850, 600, 570, 370), 15 DNA ladder marker 100bp.

Example of fingerprints resulted by microsatellites and retrotransposon based PCR methods



Example of GMO detection by qRT-PCR with the SYBR Green II

MATERIAL AND METHODS

Plant material: For analyses we used transgenic cultivar Desiree-GM/GNA and non-transgenic Desiree as control and set of available registered potato varieties respectively 152 potato varieties. Material was rendered by Potato Research Institute in H. Brod and ÚKZÚZ Lípa u Havlíčkova Brodu.

Extraction method: DNA was isolated from 100 mg of tubers or leaves using modified CTAB-PVP procedure according to Williams and Rogers and the DNA extraction trough the Invisorb Spin Plant Mini Kit (INVITEK) from tuber juice. DNA was dissolved in 100 µl of sterile water and stored in -20°C.

The Analyses were done according to the standard protocol – Biotechnological centre, Agriculture faculty, University of South Bohemia (http://www2.zf.jcu.cz/public/departments/lamb/e-amos/metod_mb.pdf)

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Abstrakt:

Cultivated potato (*Solanum tuberosum* L.) is along with wheat, rice and maize, one of the four most valuable world crops. Potato is an important food crop, as well as being widely used for livestock feeding and industrial processing as feedstock for many industrial and food applications. Currently, there are more than 4,000 different potato varieties which are cultivated in over 100 countries worldwide. The improvement and creation of new varieties with new combinations of current features or essentially new features, such as GMO potatoes or conventional varieties with better parameters of quality or resistance to biotic and abiotic factors, is one of the main goals of plant breeding. In many cases, wild allied species or “old primitive” varieties are used as donors of these features. New breeding approaches based on molecular markers allow more efficient use of these donors.

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. The Czech list of Registered Potato Varieties includes hundred and seventy-eight potato varieties in 2007. We analyzed set of available potato varieties (*Solanum tuberosum* L.) by several methods based on microsatellites and retrotransposons. Our goal of is 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”.

Genetically modified (GM) or transgenic crops, now more often called “Biotech crops” are commercially cultivated since 1996. Despite the continuing debate on biotech crops, particularly in countries of the EU, large and small farmers worldwide continue to increase their plantings of biotech crops. The global area of approved biotech crops in 2006 was 102 million hectares. In 2006, 22 countries grew biotech crops. The Czech Republic is one of the six EU countries where biotech crops are cultivated at present. The need to monitor and verify the presence and the amount of GMOs in agricultural crops and products has generated demands for analytical methods capable of detecting, identifying and quantifying either the DNA introduced or the protein(s) expressed in transgenic plants, because these components are considered as the fundamental constituents. We focused on developing of fast, precise and cheap method based on PCR to detect the presence of transgenes in potatoes – tubers and leaves, which allows monitoring the presence of GM potatoes in market, environment, etc. and to quantify “contamination” of ware potatoes (tubers) with GMOes.

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