

PCR based detection and quantification of GMO potatoes, utilization and comparison



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INTRODUCTION

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.

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 2005 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.

This study was 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 GMOs.

RESULTS

Detection by duplex – PCR:

✓ We optimized the duplex PCR for transgenic potato with the lectin gene. We used the primers GNA1 and GNA2 as a primers for lectin transgene (fragment 477 bp) and UGP primers for UDP-glucose pyrophosphorylase (UGPase) gene as a endogene.

This method is useful for identification of presence or absence of transgene, but it is not sufficiently responsive for e.g. identification of transgene contamination in food.

Detection by qRT-PCR:

✓ We optimised this method with the SYBR Green II as a detection system. We prepared the standard set for creation of standard curve. We used the two uniplex reactions in the same time and we used the same primers as for PCR duplex reaction.

This method can be used for GMO detection and above all for GMO quantification.

▪ We try to use and optimise qRT-PCR with the dual labelled fluorogenic probes or more precisely with probes labelled 5' 6-FAM/ 3'TAMRA as a detection system currently.

Detection by duplex - PCR

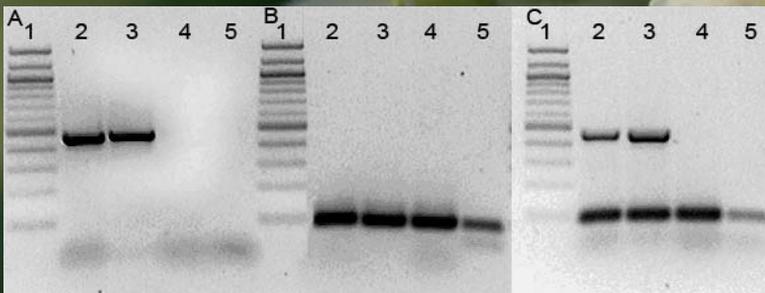


Fig.1. Example of electrophoreogram-1,5 % agarose gel. A - Uniplex PCR analyses, primers pair GNA for lectin, B - Uniplex PCR analyses, primers pair UGP for endogen, C - Multiplex PCR analyses both primers pair. Transformed Desiree DNA extracted from tuber (2) and leaf (3) and Desiree GMO free as a negative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

Detection by qRT-PCR with the SYBR Green II

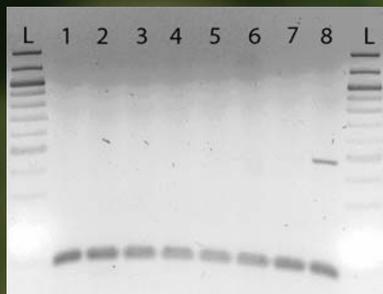
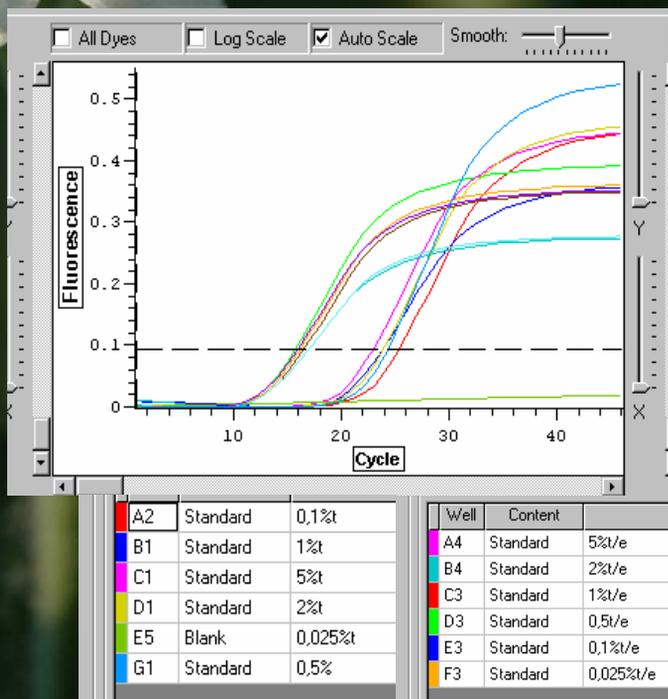


Fig.2. Example of electrophoreogram-1,5 % agarose gel. Duplex PCR analyses primers pair GNA and UGP. Transformed Desiree DNA extracted from tuber per cent of transgene: 1 - 0,1%, 2 - 0,5%, 3 - 1%, 4 - 1,5%, 5 - 2%, 6 - 3%, 7 - 4%, 8 - 5%, L - DNA ladder marker 100bp.

MATERIAL AND METHODS

Plant material: For analyses we used transgenic cultivar Desiree-GM/GNA and non-transgenic Desiree as control. GM potatoes were obtained from Potato Research Institute in H. Brod, non-transgenic tubers were obtained from Lipa u H. Brodu.

Extraction method: DNA was isolated from 100 mg of tubers or leaves using modified CTAB-PVP procedure according to Williams and Rogers. DNA was dissolved in 100 µl of sterile water and stored in -20°C.

PCR analyses: Methods for detection of lectin transgene by multiplex PCR and quantification of GM potatoes in mixture of transgenic and non-transgenic tubers by standard multiplex PCR and qPCR were developed.

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:

The need to monitor and verify the presence and the amount of GMOs in agricultural crops and products has generated a demand 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. 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. This study was focused on developing of fast, precise and cheap method based on PCR to detect the presence of transgenes in potatoes – tubers and leaves, allow monitoring the presence of GM potatoes in market, environment, etc. and to quantify "contamination" of ware potatoes (tubers) with GMones. For analyses we used transgenic cultivar Desiree-GM/GNA and non-transgenic Desiree as control. We used multiplex PCR for detection of lectin transgene qRT-PCR for quantification the amount of GMOs. We found that the techniques duplex PCR is suitable for GMO detection and we determined the detection limit. Technique qRT-PCR with the detection systems SYBER Green I. and TaqMan Probe is suitable for quantification of GMO contamination, we optimized the methodology and we determined detection and quantification limits. This study was supported by grants MSM 60076658-06, NAZV 1B44011, GACR 521/08/H042, IG 2008.