PCR BASED DETECTION OF GMO POTATOES

University of South Bohemia in České Budějovice
Faculty of Agriculture
Biotechnological Centre
Faculty of Agriculture

Nováková, A., Šimáčková, K., Bárta, J., Čurn, V.

Biotechnological Centre, University of South Bohemia, České Budějovice, Czech Republic

INTRODUCTION

Genetically modified (GM) or transgenic crops, now more often called ***"Biotech crops"*** they are commercially cultivated since 1996. And also since 1996, the first year of commercialization of biotech crops, GM potatoes were cultivated in USA, Mexico, Canada and later in South Africa, China and India. Despite the continuing debate on biotech crops, particularly in countries of the EU, large and small farmers in industrial and developing countries continue to increase their plantings of biotech crops. The global area of approved biotech crops in 2006 was 102 million hectares. Remarkably, the global biotech crop area increased more than sixty-fold in the first eleven years of commercialization, making them the fastest adopted crop technology in recent history. The rapid adoption and the commercialization of biotech crops, during the initial ten-year period 1996 to 2005, reflects the substantial multiple benefits. Beyond the traditional agricultural products of food, feed and fiber, entirely novel agriculture products will emerge pharmaceutical products including oral vaccines, special and fine chemicals. The other use of biotech crop resources can be seen in replacing non-renewable, polluting, and increasingly expensive fossil fuels.

In 2006, 22 countries grew biotech crops, 11 developing countries and 11 industrial countries. The Czech Republic is on of the six EU countries where biotech crops are cultivated at present. The most compelling case for biotechnology, and more specifically biotech crops, is their capability to contribute to: increasing crop productivity and stability of productivity and production; conserving biodiversity, as a land-saving technology; the production of renewable resource based bio-fuels.

Commercially available biotech potato cultivars are improved with regard to resistance to potato leaf roll virus (PLRV), resistance to late blight (LBR), and insects (Bt Potato). In the nineties transgenic potatoes were the fifth more cultivated biotech crop. Although at present a range of other crops is more important, biotech potatoes are cultivated in many countries and has a great potential – for food and non-food purposes.

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 GM ones.

Detection by duplex - PCR

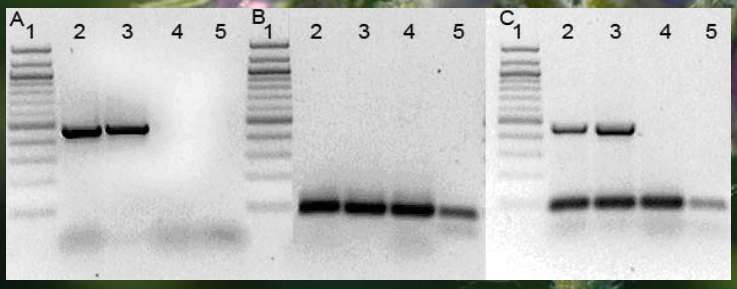

Fig.1. Example of electrophoreogram-1,5 % agarose gel. A - Uniplex PCR analyses, primers pair GNA for lectin, B - Uniplex PCR analyses, primers pair UGP for endogen, C - Multiplex PCR analyses both primers pair. Transformed Desiree DNA extracted from tuber (2) and leaf (3) and Desiree GMO free as a negative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

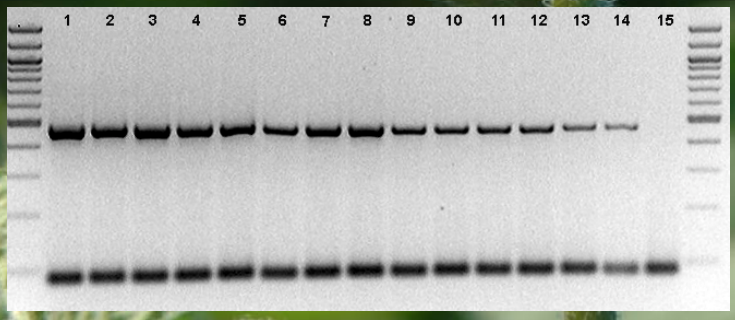

Fig.2. Example of electrophoreogram-1,5 % agarose gel. Duplex PCR analyses primers pair GNA and UGP. Transformed Desiree DNA extracted from tuber per cent of transgene (1) 100%, (2) 50%, (3) 30%, (4) 25%, (5) 20%, (6) 16%, (7) 14%, (8) 12%, (9) 11%, (10) 10%, (11) 9%, (12) 5%, (13) 2%, (14) 1%, (15) GMO free sample. DNA ladder marker 100bp.

RESULTS

Detection by duplex – PCR

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

This method is useful for identification of presence or absence of transgene, but it is not sufficiently sensitive; for example for identification of transgene contamination in food.

Detection by RT-PCR

RT-PCR with the SYBR® Green as a detection system was optimized for quantification of transgenes in potatoes. We prepare the model set of samples to create the standard curve and presence of transgenes in set of samples was quantified. We used two uniplex reactions with the same primers as were used for duplex-PCR detection.

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

Detection by RT-PCR

The standard curve

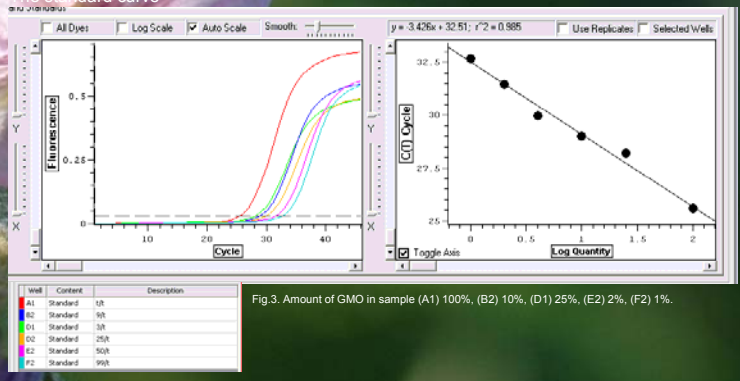

Fig.3. Amount of GMO in sample (A1) 100%, (B2) 10%, (D1) 25%, (E2) 2%, (F2) 1%.

The detection of unknown sample

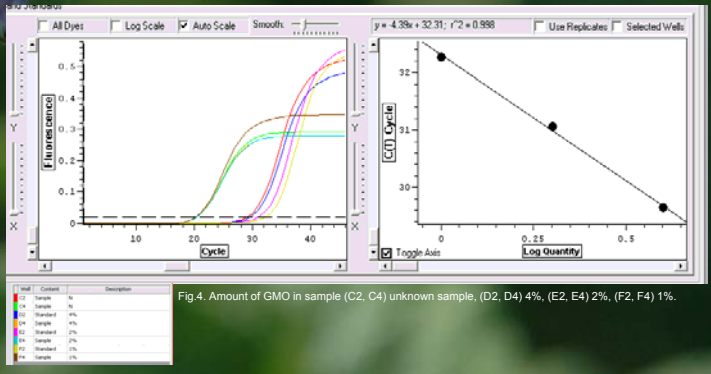

Fig.4. Amount of GMO in sample (C2, C4) unknown sample, (D2, D4) 4%, (E2, E4) 2%, (F2, F4) 1%.

CONCLUSION

The GMO detection and quantification is necessary for monitoring of GMO presence in market and environment; and for traceability of food and feed.

We used the PCR technology based detection of GMO potatoes. The duplex PCR method is useful mainly for GMO detection – for screening of absence or presence of transgene in potatoes (tubers, products, to monitor the presence of GMo in environment), but this method is not useful for GMO quantification. Real-Time PCR is useful for GMO detection and quantification, but this method is dependent on using the special equipment and this method is much more expensive than duplex PCR.

In presence, we are optimising the RT-PCR with the specific TaqMan® Probes. And we are going to use the Microarrays in collaboration with Parco Tecnologico Padano, Italy in the future.

MATERIAL AND METHODS

Plant material: Transgenic cultivar Desiree-GM/GNA and non-transgenic Desiree as control were analyzed. GM potatoes were obtained from Potato Research Institute in H. Brod, non-transgenic tubers were obtained from CISTA Lípa 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 analysis: 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)

This study was supported by grants MSM 60076658-06, NAZV 1B44011, IG 05/07.