Comparison of Different PCR-based Protocols for Detection of Roundup Ready Soybean

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Abstract: Genetically modified organisms have become a part of our environment and food chain. Roundup Ready soybean is at the moment the most frequent one that man can meet. National regulations require careful monitoring and detection of GMOs. We present in this investigation comparison of several protocols and individual steps, which are included in the whole detection procedure. Currently used CTAB based protocol is suitable for DNA isolation from the green plant tissue but also from the flour. Lectin coding sequence specific primers were suitable for soybean DNA detection unlike leu-tRNA gene specific primers. Only one of the tested CaMV sequence specific primer pairs did not amplify unspecific products under condition tested. NOS terminator specific primer pair was found suitable for routine screening. EPSPS gene was detected using two different protocols; the same primers did not identify other Roundup Ready GM crops.

Key words: GMO; Roundup Ready soybean; PCR; detection

The rapid development of biotechnology has launched genetically modified plants (GMPs) into the environment and GMPs derived products into the market. Since the techniques for plant transformation were established, tested and optimised, a wide range of applications have been investigated and performed to solve existing problems of the agriculture. Genetic modification of plants has thus been used to enhance natural plant resistance to pests and diseases or to introduce foreign DNA sequences with similar effects. Genes conferring resistance to herbicides – chemical compounds killing weeds – allow the use of new classes of herbicides that will not kill the specially developed crop. The new classes of herbicides in use decrease tillage and thus soil erosion and are considered safer for the environment because of their increased biodegradability (SHAH *et al*. 1986).

One of such widely used herbicides is Roundup®. Roundup® contains glyphosate as an active compound. In pure chemical terms glyphosate is an organophosphate in that it contains carbon and phosphorus. However, it does not affect the nervous system as organophosphate insecticides, and is not a cholinesterase inhibitor. The acute toxicity of glyphosate itself for mammals is very low. According to the World Health Organisation, the oral LD_{50} in the rat of pure glyphosate is 4.230 mg/kg (http://ace.ace.orst.edu/info/extoxnet/pips/glyphosa.htm, WAUCHOPE *et al*. 1992; LU 1995).

Glyphosate is a broad spectrum, non-selective systemic herbicide. It is effective in killing all plant types including grasses, perennials and woody plants. As an herbicide, glyphosate works by being absorbed into the plant mainly through its leaves but also through soft stalk tissue (DUKE 1996). It is then transported throughout the plant where it acts on various enzyme systems inhibiting amino acid metabolism in what is known as the shikimic acid pathway (KISHORE & SHAH 1988; STEINRUCKEN & AMRHEIN 1984). This pathway exists in higher plants and microorganisms but not in animals (LEVIN & SPRINSON 1964; STEINRUCKEN & AMRHEIN 1980). Plants treated with glyphosate slowly die over a period of days or weeks, and because the chemical is transported throughout the plant, no part survives (ANONYM 1986).

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Roundup is a total herbicide and scientists and breeders have been unsuccessful in producing glyphosate-tolerant plants using classical techniques like selection, mutagenesis or crossing. Recombinant DNA techniques have been used to confer glyphosate tolerance to a variety of crop plant species. They are known as Roundup Ready cultivars (MONSANTO 1997) and they are known as genetically modified plants (GMPs).

In Europe EC directives and national laws drive the GMPs handling. Precautionary principle was accepted in EC. Labelling and traceability of GMPs and transgenes containing food products is required (EU directives). Similar laws were accepted in the Czech Republic – Law 153/2000 regulates the release of GMPs into the environment and 306/2000 Sb. regulates the use of GMPs in the food chain. Careful monitoring of GMPs and derived products is also required to protect consumers and environment. Therefore robust, reliable and sensitive techniques are required for these purposes. First comprehensive EU guidelines for the detection of proteins and nucleic acids in genetically modified organisms (GMO), used for human and animal feeding was published in 1999 (BERTHEAU 1999).

There are two main approaches considered for GMPs and derived products detection: (1) specific protein detection using e.g. ELISA (enzyme linked immunosorbent assay), suitable for analysis of raw material and (2) specific DNA sequence detection (GACHET *et al*. 1998; DUIJN *et al*. 1999; LIPP *et al*. 2001). The polymerase chain reaction (PCR) is a highly specific and sensitive method for detection of small amount of nucleic acid (MULLIS & FALOORA 1987; MEYER *et al*. 1996). Generally, two approaches are used. Transgens introduced into plants consist of regulation sequences recognised by plants and specific gene(s). Usually, GMPs, which are in use nowadays, contain 35S CaMV (small subunits of cauliflower mosaic virus) promoter, which is a strong constitutive one and NOS (Nopalin synthase) terminator from *Agrobacterium* plasmid. These sequences are used for routine screening (LIPP *et al*. 1999a, b). Complementary, it is also possible to detect coding sequence of the transgene itself. This approach is often used in case of Roundup Ready soybean (PASQUALONE 2000).

In this study we present the performance testing results of several protocols using different matrices including reference standardised sample material for detection of Roundup Ready transgene in soybean. Commercial detection kit is also included.

MATERIALS AND METHODS

List of analysed materials: CRM IRMM standards (Certified Reference Materials – Institute for Reference Materials and Measurements, Geel, Belgium) (Fluka 310R-soybean powder standard set), Roundup Ready soybean (obtained as a commercial lot), leaves of Roundup Ready wheat and Bt corn (kindly provided by Monsanto), transgenic potatoes (developed in RICP Prague), non modified soybean cultivar (Monsanto), non modified cultivars of barley, corn, potato, rapeseed, sugar beet and wheat. Seeds were cultivated in the pots and plants were harvested after 2 weeks and stored at –80°C upon further processed. Also soybean flour was used being prepared from soybean kernels using a mill and a 100 µm sieve.

DNA isolation: DNA was isolated from soybean leaves, soybean seedlings, grounded soybean powder standard set, soybean flour and leaves of other above mentioned species. Three basic protocols were tested: (1) The procedure following the methods described in the German Official Collection of Test Procedures using commercial kit Hanse Analytik (CTAB – cetyl-trimetyl-amonium-bromide method), (2) protocol according to SAGHAI-MAROOF *et al*. 1984 (CTAB method) briefly: 1–2 g of plant material was carefully grounded into the fine powder, incubated in CTAB buffer at 65°C, proteins were degraded by proteinase K and cell debris was removed by centrifugation after chloroform extraction, DNA was precipitated from aqueous phase by isopropanol, ribonucleic acids were degraded by RNase, (3) mini-preparation from 100 mg of tissue was performed using DNeasy Plant Mini Kit (Quiagen, Plant Mini Kit Handbook, product No. 69104), procedure is based on separation of DNA using silicagel membrane, (4) additionally DNA was purified using WIZARD DNA clean-up system (Promega, Cat. No. A7280).

Specific detection of soybean internal gene: Three primer combinations were tested to identify the presence of soybean DNA – (1) specific leu-t-RNA gene and (2) soybean lectin gene.

(1) leu-t-RNA (leyucyl t-RNA) primers (provided kindly by ISCI Fiorenzuola d'Arda, Italy)

5′-ATT gCA gCA TTC TTC ggA ggA-3′

5′-ACT ACT ggT TTg TTg Aag Aag C-3′

were used under following conditions: Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 50 ng of template DNA and 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystems). Ampli Taq Gold was used in all experiments described in this work. Amplification was performed in a PE thermal cycler 9600 using following cycling: 12 min 92°C initial denaturation, followed by 40 cycles of 30 s at 95°C, annealing 30 s at 60°C, 30 s extension at 72°C and final extension 10 min at 72°C.

(2) Protocol suggested by PIETSCH *et al*. (1997) was used to amplify a part of lectin gene. Primer sequences were designed as followed:

5-GAC gCT ATT gTg ACC TCC TC-3

5-TgT Cag ggg CAT AGA Agg Tg-3

Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystem) and 50 ng of template DNA. Amplification was performed in a PE thermal cycler 9600 using following cycling: 12 min 92°C initial denaturation, followed by 40 cycles of 30 s at 92°C, annealing 30 s at 60°C, 30 s extension at 72°C and final extension 10 min at 72°C.

Detection of CaMV promoter sequence: CaMV promoter sequence was amplified using two approaches.

(1) Primers (VOLLENHOFER *et al*. 1999) were designed as follows:

5′-CCg ACA gTg gTC CCA Aag Atg gAC-3′

5′-ATA Tag Agg Aag ggT CTT gCg AAg g-3′

Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystems) and 50 ng of template DNA. Amplification was performed in a PE thermal cycler 9600 using: 12 min 92°C initial denaturation, followed by 40 cycles of 1 min 95°C, annealing 30 s at 66°C, 30 s extension at 72°C and final extension 10 min at 72°C.

(2) Primers suggested by LIPP *et al*. (1999b):

5′-gCT CCT ACA AAT gCC ATC A-3′

5′ -gAT AgT ggg ATT gTg CgT CA-3′

Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystem) and 50 ng of template DNA. For amplification was used a PE thermal cycler 9600 12 min 92°C initial denaturation, followed by 40 cycles of 1 min 95°C, annealing 30 s at 66°C, 30 s extension at 72°C and final extension 10 min at 72°C.

Detection of Roundup Ready soybean: Two protocols were tested:

(1) Primer sequences proposed by VOLLENHOFER *et al*. (1999) were used

5′-TCA TTT CAT TTg gAg Agg ACA Cg-3′

5′-ggA ATT ggg ATT Aag ggT TTg TAT C-3′

Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystems) and 50 ng of template DNA. Amplification was performed in a PE thermal cycler 9600 using following cycling: 12 min. 92°C initial denaturation, followed by 40 cycles of 1 min T 95°C, annealing 30 s at 62°C, 30 s extension at 72°C and final extension 10 min at 72°C.

(2) Detection of the Roundup Ready soybean DNA using the commercial kit Gene Check (Hanse Analytik). The detection procedure follows the methods described in the German Official Collection of Test Procedures. One control reaction and one specific reaction are carried out. In the control reaction a part of the lectin gene typical for native and genetically modified soybean is amplified and helps verify accordance with the test parameters. The specific reaction is based on amplification at the point of transition of the 35-S-promoter and the transit peptide-sequence (originated from *Petunia*).

Detection of NOS terminator: NOS terminator was detected using protocol of VOLLENHOFER *et al*. (1999). Primer sequences were as followed:

5′-gAA TCC TgT TgC Cgg TCT TgC gAT g-3′

5′-TCg CgT ATT AAA TgT ATA ATT gCg ggA CTC-3′

Reaction mixture (total volume 25 µl) consisted of 1× buffer supplied with Taq polymerase, 2.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM of each primer, 1.25 U Ampli Taq Gold DNA polymerase (Applied Biosystems) and 50 ng of template DNA. PE thermal cycler 9600 was used for the amplification. Amplification profile consisted of 12 min. 92°C initial denaturation, followed by 40 cycles of 1 min T 95°C, annealing 30 s at 68°C, 30 s extension at 72°C and final extension 10 min at 72°C.

Detection of CaMV virus: CaMV virus can be detected according to WOLF *et al*. (2000). Two primer pairs were used:

(1) 5′-gCg TAY ACA ACA AgT Cag CAA ACA-3′
5′-TCC Tgg AgA TTA TTA CTC ggg TAg A-3′

(2) 5′-CCA gAA gAA CAT Tgg gTC AAT gC-3′
5′-ATA gCT gAC AgA AgT TgT TgC Cg-3′′

Reaction conditions were used as described by authors except for Ampli Taq Gold and PE thermocycler.

RESULTS AND DISCUSSION

Reliable protocols are required for routine detection of GMOs and their parts in the environment and food chains. Whereas protein analysis allow detection of GMOs only in non-processed material, pieces of DNA can be extracted from any matrices. After, GMO specific DNA can be detected. PCR based approach is considered very suitable for detection of a specific DNA sequence (GADANI *et al*. 2000). Protocols consist of several steps – DNA isolation, DNA quality verification, targeted sequences amplification and products analysis. Up to now, EC have not issued validated protocols for GMO detection up to now. We compared results obtained by Hanse Analytik detection kit and protocols currently recommended in literature, which are cheaper to perform.

DNA quality and quantity are important for successful DNA analysis. Generally, Taq polymerase does not require high quality DNA. Sometimes even direct templates like a piece of leaf tissue could be used (PECCHIONI *et al*. 1996; MARTYNKOVÁ *et al*. 1997). However, some compounds could be efficient inhibitors of this enzyme (INIS *et al*. 1995). Therefore first we compared

several methods of DNA isolation and purification. Three basic protocols were used to prepare DNA from different matrices (leaf tissue, young seedlings and soybean flour). Fresh leaf and seedling tissues are usually easy matrices for DNA isolation, soybean flour can be rich in some types of polysaccharides. (1) Hanse Analytik kit yielded a low amount of DNA in comparison with SAGHAI-MAROOF *et al*. (1984) protocol, especially in the case of flour. More over RNA is not removed during the Hanse Analytik procedure, so that subsequently higher primer concentrations may be required later for amplification. Subsequent purification of DNA using Wizard (Promega) did not substantially improved the DNA quality (Table 1 and Fig. 1). RNaseA is used to remove RNAs from the nucleic acid extract according to SAGHAI-MAROOF protocol (2). Usually, more then 500 µg of DNA could be isolated from 2 g of leaf tissue or seedlings (Table 2). The protocol is suitable for isolation of DNA from fresh or frozen leaf tissue and young seedling. The DNA quality was satisfactory as verified by agarose electrophoresis and spectrophotometry ($A_{260/280} \leq 1.6$). When DNA from flour was isolated, chloroform extraction had to be repeated twice to remove proteins and to reach similar DNA quality as in the case of leaf tissue and seedlings. As a consequence of repeated chloroform extraction step the DNA yield was slightly lower. Even though the both Hanse Analytik and SAGHAI-MAROOF *et al*. (1984) protocols are based on the same principle – a selective precipitation of nucleic acid in the CTAB and NaCl solution, the yield was substantially different. Hanse Analytik kit is suited for isolation of DNAs from different matrixes and is not probably suited to get maximum yield. DNeasy (3) isolation kit (Quiagen) is intended for isolation of DNA from small amount of tissue (approximatelly100 mg). The total DNA yield – 1500 ng was stable in the case of all the three matrices. The purity of isolated samples was also comparable (A_{260}/A_{280} = 1.8–1.9). From the point of view of DNA quality, which can be detected spectrophotometrically and after electrophoretic separation, all the three methods are comparable. Hanse Analytik protocol, however, does provide substantially lower amount of DNA.

The other, most important check of DNA quality the amplification of internal gene is. To do this check, a short string up to 200 pb of the targeted gene is usually amplified. The size is chosen because highly degraded DNA can be isolated from processed products and the protocol should be applicable for analysis of DNA isolated from different matrices. We tested the primer pairs (1) provided by Hanse Analytik (Fig. 2), (2) specific for the lectin gene PIETSCH *et al*. (1997) (Fig. 3) and (3) leu-tRNA gene (V. Terzi, ISCI Fiorenzuola D'Arda (Italy) – personal comm.).

Table 1. Comparison of DNAs quality after their isolations by Hanse Analytik kit and after their subsequent purifications by Wizard kit. First number in the column – Hanse Analytik kit, second number indicates the quality after purification by Wizard protocol. Ratios of absorbance (A) at different wavelength (in nm) indicate presence of impurities

Matrices	$A_{260/280}$	$A_{260/230}$
Leaves	1.74 ›1.69	2.41 › 2.75
Seedlings	1.94 › 1.98	2.22 › 2.26
Flour	0.94 › 1.2	0.97 › 1.23

Table 2. Quantity and quality of soybean DNAs isolated using Commercial kit Hanse Analytik (HA) and Saghai Maroof protocol (SM) from 2 g of different matrices

Matrices	Average yield of DNA	$A_{260/280}$
Leaf – HA	500 ng	1.74
Leaf – SM	400 mg	1.5
Seedling/HA	200 mg	1.94
Seedling/SM	500 mg	1.6
Flour/HA	50 ng	0.94
Flour/SM	100 mg	1.4

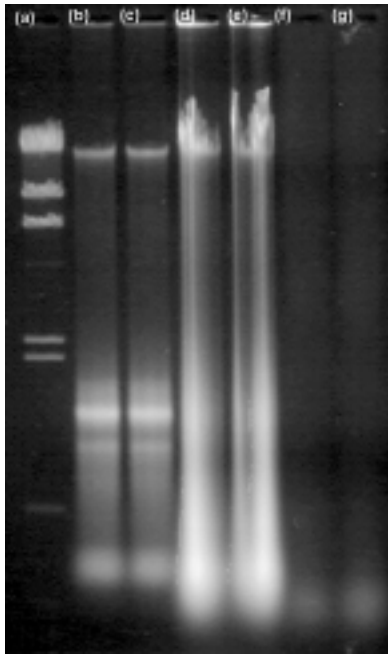

Fig. 1. DNA isolated from soybean leaves (b, c), young seedling (d, e) and flour (f, g) using Hanse Analytik kit. DNA ladder l/HindIII (MBI Fermentas) lane (a) was used beside spectrophotometrical evaluation to estimate DNA concentration

Protocol proposed by Hanse Analytik (1) identified soybean DNAs; however, unspecific products could be detected after PCR reactions, which do not contain soybean DNA (Fig. 2). Lectin specific customer made (2) primers identified all soybean DNA containing samples. No products – specific or unspecific were detected when corn, potato, wheat, rapeseed, barley or sugar beet DNAs were used as the templates (Fig. 3). Leu-tRNA gene (3) specific primer pair did amplify the products from various templates, so this one can be used for verification of the presence of different plant DNAs, not for detection of soybean DNA specifically. Leu-tRNA is a housekeeping, conservative gene, so that homology between different species could be rather high KINOUCHI *et al*. (2000). We conclude, that lectin-specific primer pair designed by PIETSCH *et al*. (1997) is highly specific and it is possible to use it successfully for identification of soybean DNA.

CaMV promoter and NOS terminator sequences are commonly used for routine GMP screening. This screening step is not compulsory included in the Hanse Analytik protocol. So that only two different customer made primer pairs were tested for CaMV promoter detection. The first (VOLLENHOFER *et al*. 1999) one amplifies specifically CaMV sequence not only in soybean, but also in transgenic potato and transgenic Bt corn. In addition, the primer pair amplifies corresponding virus sequence, too. The virus DNA was purchased from German National Laboratory. The second tested primer pair (LIPP *et al*. 1999a, b) amplified also some unspecific products under reaction conditions tested. This primer pair is therefore not suitable for routine screening or the protocol needs to be better optimised (thermocycler type, composition of reaction mixture, PCR polymerase types, etc).

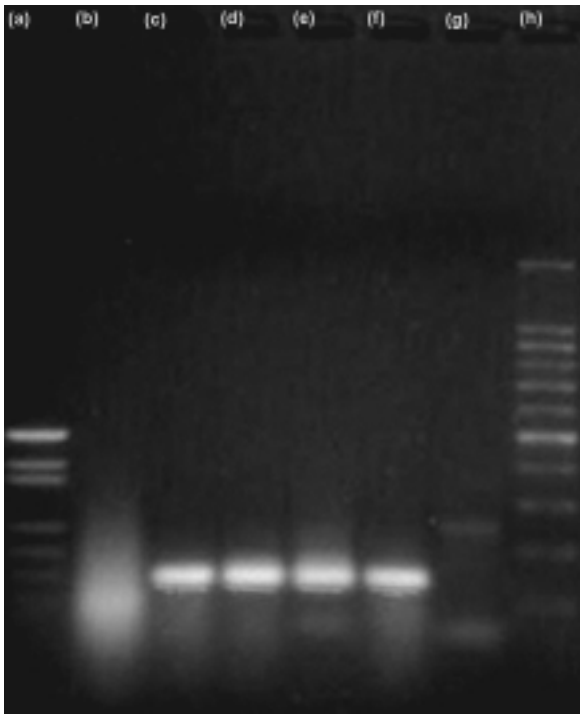

Fig. 2. Detection of the lectin gene in soybean DNA (a) DNA ladder. (b) reaction control, (c) leaf DNA, (d) seedling DNA, (e) flour DNA, (f) positive control – Hanse Analytik, (g) template-free, (h) DNA ladder

It was shown, that some matrices might contain CaMV virus itself. CaMV virus can contaminate especially vegetable containing samples. (LIPP *et al*. 1999a, b;

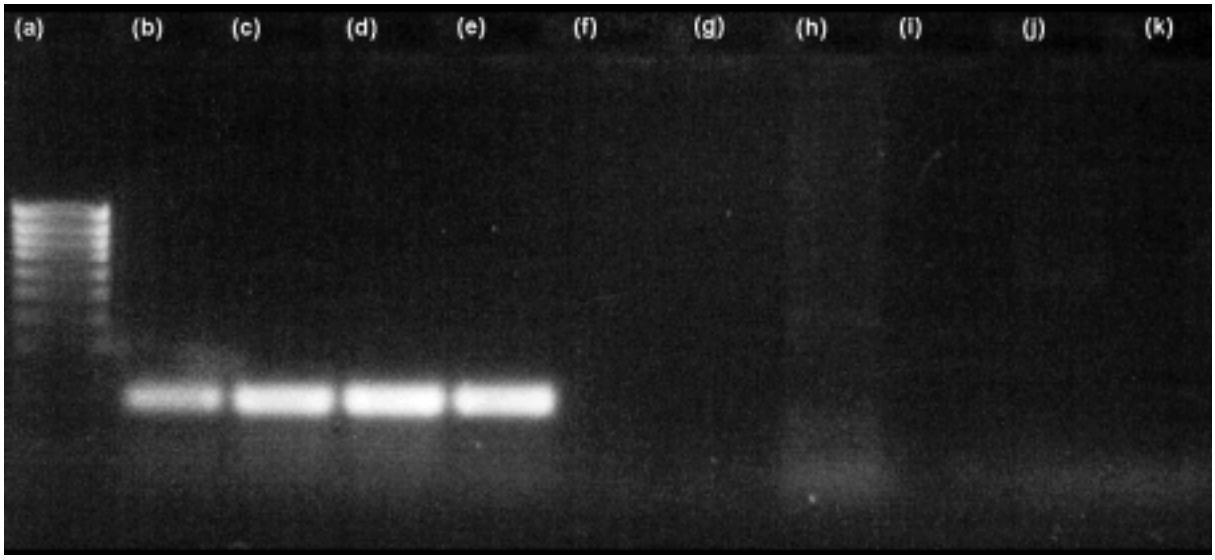

Fig. 3. Detection of soya lectin gene using PIETSCH *et al*. (1997) primer pair: left to right DNA ladder, non-modified soybeans (IMMR standard) , GM soybeans (0.1% IMMR standard), GM soybeans (0.5% IMMR standard), GM soybeans (2.0% IMMR standard), sugar-beat leaf, wheat leaf, corn flour, corn leaf, non modified corn IMMR standard, template-free control

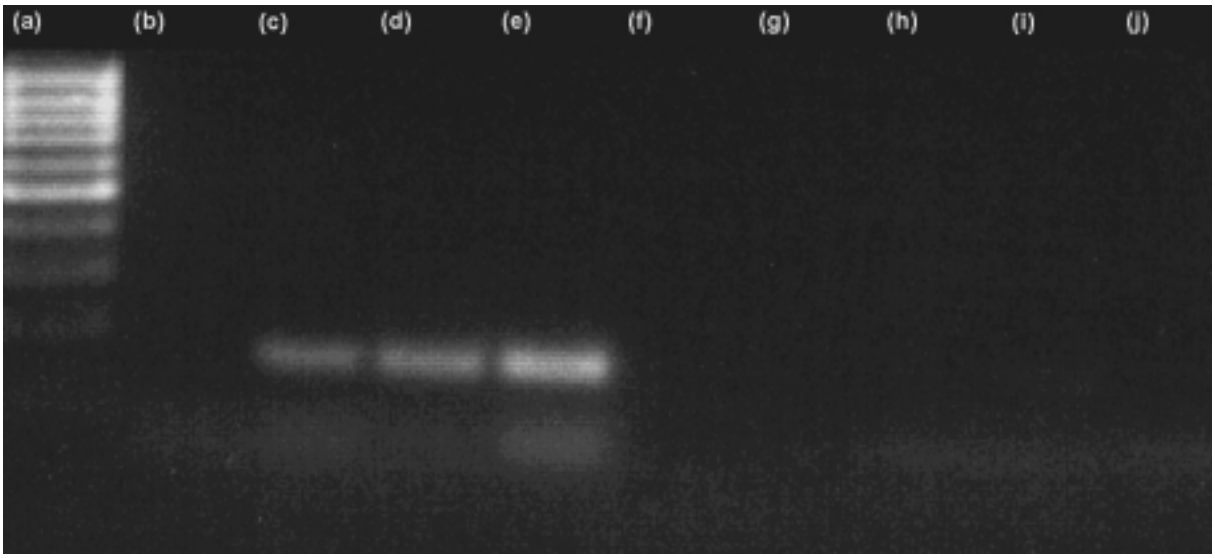

Fig. 4. Detection of CaMV promotor using VOLLENHOFER *et al*. (1999) primer pairs: left to right DNA ladder, non-modified soybeans (IMMR standard), GM soybeans (0.1% IMMR standard), GM soybeans (0.5% IMMR standard), GM soyabeans (2.0% IMMR standard), non-modified soya leaf, sugar-beat leaf, wheat leaf, corn flour, corn leaf, template-free control

WOLF *et al*. 2000). The combined results from 27 laboratories indicated that the probability of false positive results is on average only about 1% for soybeans (LIPP *et al*. 1999a, b), however nonzero. To posses a tool applicable to exclude possible false positives another primer pair was additionally used. We did not detect virus particles in any analysed sample.

Only one NOS terminator specific primer pair was tested. Products of expected size were amplified using RR soybean DNA, transgenic potato and corn. No products were amplified when non-modified corn, potato, wheat, rapeseed, barley or sugar beet DNAs were used as the templates.

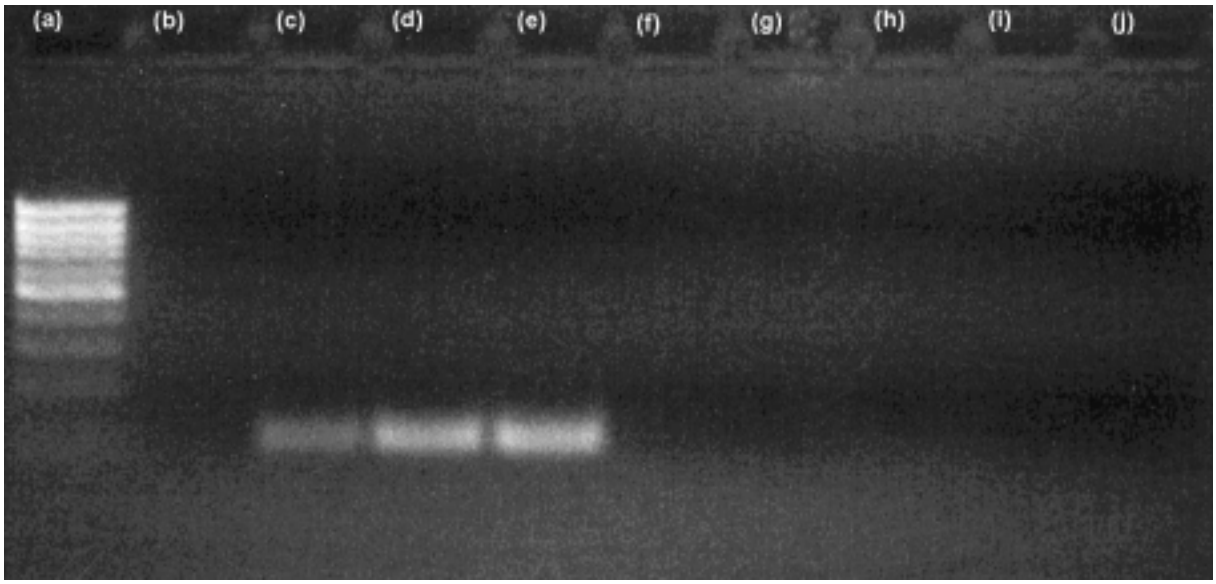

Fig. 5. Detection of RR soybean transgene using VOLLENHOFER *et al*. (1999) primer pairs: left to right (a) DNA ladder, (b) non-modified soybean (0% CRM IRMM standard), (c) GM soybean (0.1% CRM IRMM standard), (d) GM soybean (0.5% CRM IRMM standard), (e) GM soybean (2.0% CRM IRMM standard), (f) non-modified soybean leaf, (g) sugar beet leaf, (h) wheat leaf, (i) corn leaf, (j) template-free control

Two EPSPS specific primer pairs were tested. (1) Primer pair from Hanse Analytik kit, sequence of which is not known, and (2) primer pair designed by VOLLENHOFER *et al*. (1999). In the both cases forward primer was complementary to a sequence of transit peptide and reverse primer fitted to EPSPS coding sequence. EPSPS enzyme, the target of glyphosate action, is synthesised in the cytoplasm and then transported to the chloroplast (KISHORE & SHAH 1988). The translocation of the protein to the chloroplast is carried out by an N-terminal protein sequence called the chloroplast transit peptide (CTP) (DELLA-CIOPPA *et al*. 1986, 1987).

It is known, that limit (L) L_{theor} for the detection of Roundup Ready soybean DNA with the PCR set-up applied can be 0.005% genetically modified organism (GMO/non-GMO – w/w) that corresponds to 30 copies of the GMO soybean genome per single PCR reaction. In pre-mixed powder preparations of soybean it is possible to detect 0.1% GMO/non-GMO (w/w), i.e. this is the L_{prac} (JANKIEWITZ *et al*. 1999). Roundup Ready soybean and CRM IRMM standards (0.1, 0.5, 1, 2 and 5% of transgenic RR soybean [w/w]) were therefore used to test the sensitivity of the reaction. It was possible to amplify the target sequence from 50 ng of DNA containing 0.1% of transgenic DNA (50 pg) without modification of the protocol. To check the sensitivity of the reaction 1% standard was also diluted 1:1, 1:5, 1:10 and 1:20 with non-transgenic corn DNA. Again, the target sequence was amplified in all the cases, it means, that it was possible to amplify target sequence from 25 pg of transgenic DNA.

Both the primer pairs identify reliably RR soybean. No product was detected in RR corn or RR using these primers. In fact, three genes which provide field-level tolerance to glyphosate, the active ingredient in Roundup® herbicide, have been introduced into commercial cultivars. The first glyphosate-tolerant EPSPS gene was isolated from a soil bacterium, *Agrobacterium* (BARRY *et al*. 1994; DUKE 1996). Recently, the EPSPS gene from corn (*Zea mays*) has been mutagenised *in vitro* to obtain a glyphosate-tolerant enzyme. And this enzyme is 99.3 % identical to the parent enzyme (MONSANTO 1997). Also, a gene that encodes for a glyphosate-degrading enzyme called glyphosate oxidoreductase (GOX) was isolated from *Achromobacter* strain LBAA, a soil bacterium ubiquitous in nature (BARRY *et al*. 1994). The encoded enzyme deactivates the herbicidal effect of glyphosate. More over, in order to achieve efficient expression of bacterial genes within plants, it has been common for researchers to modify the codon usage pattern of genes of bacterial origin prior to introducing them into plants (CROON 1996). So that it is difficult to use the same protocol for the detection of the gene conferring herbicide resistance in other plant species. No products were amplified across different non-modified species – corn, potato, wheat, rapeseed, barley or tomato. We can conclude, that these primers are highly specific and probably can discriminate Roundup Ready soybeans (Monsanto) only.

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Souhrn

OVESNÁ J., DĚDIČOVÁ L., HORÁČEK J., SADILOVÁ E., KUČERA L., MĚSKOVÁ L. (2002): **Porovnání několika metod založených na PCR pro detekci Roundup-Ready sóji**. Czech J. Genet. Plant Breed., **38**: 55–63.

Geneticky modifikované organismy se stávají součástí našeho přírodního prostředí a potravního řetězce. Nejrozšířenější je v současné době Roundup Ready sója. Zákony jednotlivých zemí vyžadují pečlivé monitorování rozšíření GMO v prostředí

a jejich částí v potravním řetězci. V předkládané publikaci porovnáváme jednotlivé kroky, které jsou částmi několika postupů detekce. Běžně používaný postup izolace DNA založený na selektivní precipitaci v prostředí CTAB se ukázal vhodným nejen pro izolaci DNA ze zelených pletiv, ale také z mouky. Na rozdíl od primerů amplifikujících sekvenci leu-tRNA genu je PCR detekce přítomnosti genu pro lectin naprosto specifická. Pouze jeden z testovaných primerových kombinací pro amplifikaci 35S CaMV promotoru neamplifikoval za testovaných podmínek nespecifické produkty. Primerový pár detekující specificky sekvenci NOS terminátoru byl shledán vhodným pro rutinní detekci. Gen kódující protein EPSPS byl amplifikován podle dvou odlišných protokolů. Tytéž primerové páry neidentifikovaly jiné GM plodiny obsahující genetickou modifikace se stejnou funkcí.

Klíčová slova: GMO; Roundup-Ready sója; PCR; detekce

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