MERGING PROCEDURES FOR DETECTION OF SOY DNA AND PRESENCE OF GM SOY IN FOOD SAMPLES

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ABSTRACT

The use of genetically modified soy is becoming more and more prevalent and the detection of these products becomes an obligation for the food suppliers and food importers as well. Republic of Macedonia creates a legal frame for GMO that is in correspondence with the EU Regulations. Therefore the GMO detection methods are used according to this legislative. This study reports the screening of raw soy and some products that contain soy in a single step with duplex PCR. Few years ago the screening was performed in two steps, one for revealing the soy DNA, and the second for detecting the presence of the construct that is present in GM soy. An optimization of the PCR conditions was performed focusing on $MgCl_2$ concentration and primers annealing temperature. The achieved data shown that concentration of 2,5mM $MgCl_2$ and temperature of 60ºC are appropriate to amplify the both fragments in a single reaction. The results did not show any false positive or false negative data. They were well-matched with those from the separately accomplished reactions. This kind of doubled PCR enable faster detection of the presence of GM-soy. The method is shorter and cheaper. It gives a possibility to eliminate so many negative samples before the quantification step with real-time PCR.

Keywords: GM soy, duplex PCR

Type of contribution: Poster presentation

INTRODUCTION

Due to the fact that the nucleotide sequence of GMOs is well known, the detection is done effectively using PCR.

In order to shorten the costs in this study we did the screening of raw soy and some food products that contain soy in a single step with duplex PCR. The screening of RRS before was performed in two steps, one for revealing soy DNA, and the second for detecting the presence of the construct that is present in GM soy.

MATERIALS AND METHODS

Samples of different food products that contain soy and unprocessed soybeans as well were involved in this study.

Due to the single authorized modification of soybean the standard procedure for detection of GM soy includes two PCR reactions, the first for affirmation the successful soy DNA extraction, and the second for persuasion the presence of a part from a construct of GM soy (3).

The soy DNA was detected by PCR for amplification of the part of the lectin gene. The used primers produced by Roth, GM03 (5' - GCC CTC TAC TCC ACC CCC ATC - 3') and GM04 (5' - GCC CAT CTG CAA GCC TTT TTG TG - 3') amplified a fragment with 118bp in length. Reactions contain 3,0 mM $MgCl_2$; 0,4 mM dNTP; 0,2 pM GM03/GM04, 1U *Taq* Polymerase and 100ng DNA. The annealing temperature was at 60°C.

The modified DNA was detected with the second pair of primers RRS1 F (5'-CAT TTG GAC AGG ACA CGC TGA-3') and RRS1 R (5'-GAG CCA TGT TGT TAA TTT GTG CC-3'), produced by IDT (Integrated DNA Technologies, IA, USA). The amplicon has length of 74 bp comprehend the end part of 35S promoter and initial part of the CTP4 inserted gene (Fig.1).

The reactions contain 2,5 mM $MgCl_2$, 0,4 mM dNTP, 0,2 mM RRS1-F/RRS1-R and 0,5U Ampli*Taq* Gold Polymerase activated with hot-start. The annealing temperature was at 60°C.

The concentration of $MgCl_2$ was optimized by changing it in range from 0,5 mM up to 4,5 mM. Optimization was done also for the annealing temperature, in order to find appropriate value for annealing the two pairs of primers, one on the 35S – promoter and the second on the CTP4 inserted gene form petunia.

RESULTS AND DISCUSSION

The results from the $MgCl_2$ optimization were visualized on 2.5% agarose gel. The samples with 0.5mM and 1.0mM $MgCl_2$ did not show any fragments, while in the samples with 1.5mM and 2.0mM $MgCl_2$ a weak signal was registered. The samples that had concentration of $MgCl_2$ form 2.5mM up to 4.5mM show visible and clear fragments. Due to the fact that the higher $MgCl_2$ concentration results usually with unspecific amplification, we took the 2.5mM $MgCl_2$ as the most suitable concentration for these reactions (Fig. 2). Optimization of the temperature was done with 12 different values in the range from 55°C to 65°C (Fig. 3).

The analysis on a 2.5% agarose gel show that the strength of the signal started to be weaker at the temperature above 62.2°C (#11 and #12). The rest of the samples that were amplified at

values between 55.8°C and 62.2°C did not show any differences in the bands that is an indicator for the possibility for primers annealing at the temperatures with a wider range.

Due to the similar conditions in both reactions we tried to perform a duplex PCR with both pairs of primers, one for the amplifying the part of the lectin gene, end the second pair that is used to prove the presence of the insert. So, the single tube contains 2,5 mM $MgCl_2$, 0,4 mM dNTP, 0,2 mM primers (GM03/GM04 and RRS1-F/RRS1-R), 1,0U Ampli*Taq* Gold Polymerase and 100ng of soy DNA. The program was identical with the one for amplification of GM soy.

The annealing temperature of 60°C and the needed concentration of 2,5 mM $MgCl_2$ was the reason to our essay to amplify both fragments at once. This double PCR enable faster detection of GM soy. The method is cheaper and shorter. The results that were gained through the experiment did not show false positive or false negative values. Just in one case was detected non-specific fragment that has an unexpected length and this result was rejected from the analyses. The comparison of all other results from the duplex PCR did not show any differences with the results of the same samples, but analyzed separately in two isolated reactions. The verification was done by agarose gel electrophoresis (Fig. 4).

The positive control (#4) and the samples that contained DNA derived from the expression box resulted with two fragments of 118 and 74 bp. The negative control and the samples that were not genetically modified resulted with just one fragment of 118 bp from the lectin gene. The blind did not show any fragment that was a prove for the absence of any contamination and exclude any possibility of false positive results.

CONCLUSIONS

Merging these procedures enabled faster detection of the GM-soy presence. It gives a possibility to eliminate so many negative samples before the quantification step with real-time PCR will be taken. The results did not show any false positive or false negative data. They were well-matched with those from the separately accomplished reactions.

REFERENCES

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Figures

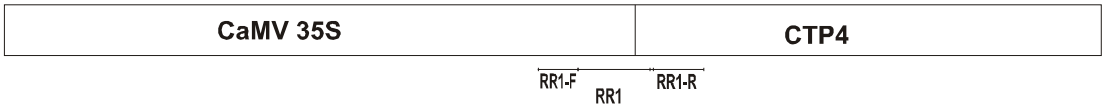

Figure 1. Amplicon that prove the presence of RRS

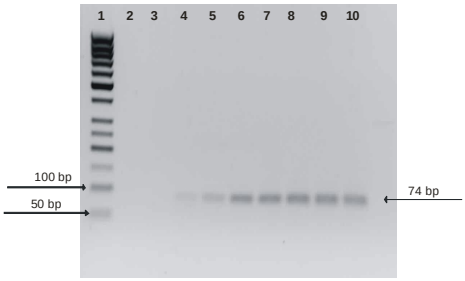

Figure 2. $MgCl_2$ gradient concentration from 0.5 Mm to 4.5 mM
Amplicons of 74 bp of the same sample on a 2.5% agarose gel. #1 – 50 bp ladder, #2 - 0,5 mM $MgCl_2$, #3 - 1,0 mM $MgCl_2$, #4 – 1,5 mM $MgCl_2$, #5 – 2,0 mM $MgCl_2$, #6 – 2,5 mM $MgCl_2$, #7 – 3,0 mM $MgCl_2$, #8 – 3,5 mM $MgCl_2$, #9 – 4,0 mM $MgCl_2$ and #10 – 4,5 mM $MgCl_2$.

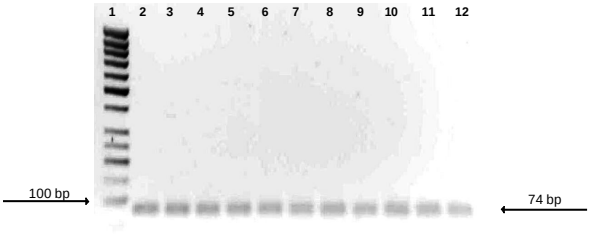

Figure 3. Temperature gradient form 55°C to 65°C. Electrophoregram of 2.5% agarose gel. #1 - 50 bp ladeer; (#2) 55,8 °C, (#3) 56,3 °C, (#4) 57,1 °C, (#5) 58,1 °C, (#6) 58,8 °C, (#7) 59,6 °C, (#8) 60,2 °C, (#9) 61,6 °C, (#10) 62,2 °C, (#11) 63,3 °C и (#12) 64,2 °C.

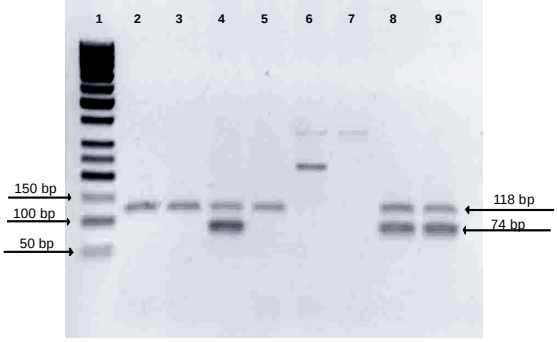

Figure 4. Duplex PCR results of 2.0% agarose gel electrophoresis.
#1 - 50 bp Size ladder; #2 - soybeans, #3 - soybeans / 2, #4 – positive control, #5 – negative control, #6 – soymilk (unspecific amplification), #7 – Blind, #8 – chicken frankfurter, #9 – chicken frankfurter / 2