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Fluorescence based method for specific sequence detection of genetically modified tomato

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Abstract

In recent years genetic engineering resulted to the production of genetically modified organism (GMO) included many important crops. Several attempts have been focused on development of methods for detection of genetically modified organisms (GMOs). In this study fluorescence based method for specific sequence recognition of plant CaMV promoter in transgenic tomato was developed which was upon hybridization of specific probe and amplified targets from transgenic tomato plants. Methylene Blue (MB) as a fluorescence marker has been used and showed quenching effect after addition of different concentration of target ssDNA to the solution. After optimizing of environmental conditions the results showed that decreased fluorescence was proportional with the concentration of 3×10^{-9} to 1×10^{-7}M of target ssDNA with the detection limited of 2×10^{-9} M.

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Introduction

Production of genetically modified organisms (GMOs) includes introduction of recombinant DNA into the plant genomes which expresses novel proteins conferring the desired characteristics. (Levandi *et al.*, 2008). The GMOs have some advantages, such as improved nutritional properties and resistance to plant disease (Koziel *et al.*, 1993; Gao *et al.*, 2000). Because of ethical issues and also environmental risk and biosafety , development of these GMOs require regulation and labeling of grains and foodstuff that contain GMOs and testing of these products. Also there is very strong motivation for detecting of GMOs in order to ensure of effectiveness of genetic engineering methods .Several screening methods includes detecting of novel protein or new DNA has been reported. Most of protein detection methods are based on enzyme- linked immunosorbent assay (ELISA) which is so complicated, labor- intensive and expensive. Higher stability of DNA rather than protein makes it profitable alternative analyte for detection process. Hence most of studies have focused on methods such as polymerase chain reaction (PCR) (Xu *et al.*, 2006; Brodmann *et al.*, 2002; Huang and Pan, 2004), microarray (Germini *et al.*, 2004), electrochemistry (Ahmed *et al.*, 2009) and optical methods (Qiu *et al.*, 2013).

Polymerase chain reaction (PCR) is the most frequently used method for GMOs detection due to its high sensitivity and stability but requirement of professional design and intensive operations and also its cost and time consuming still restricts its application. So it is necessary to explore a simple method to detect GMOs (Qiu *et al* ., 2013). Therefore, new analytical methods which can handle simple and rapid detection of GMOs are urgently needed.

Genetically molecular nanobiosensors those transduce the interaction of a target molecule (e.g., DNA) with a recognition element into a macroscopic observable signal provide a tool capable of solving (Lalonde *et al.*, 2005).

One of the methods for detection of specific DNA sequence is based upon the DNA hybridization between single-stranded target DNA and its complementary single strand, which is possible through denaturing target dsDNA before the experiment. However, since dsDNA is the natural structure of genome, sequence-specific recognition of dsDNA may be used to recognize the transferred gene into genome directly.

The majority of crop plant constructions for herbicide or disease resistance employ a Promoter from cauliflower mosaic virus (CaMV). Regardless of the gene transferred, all transfers require a promoter, which is like a motor driving production of the genes' message. Without a promoter, the gene is inactive, but replicated, CaMV is used because it is a powerful motor which drives replication of the retrovirus and is active in both angiosperms and gymnosperms.

CaMV has two Promoters 19S and 35S, of these two the 35S promoter is most frequently used in biotechnology because it is most powerful. The 35S promoter is a DNA (or RNA) sequence about 400 base pairs in length.

Generally dsDNA has affinity to bind to various aromatic compounds through different binding sites (King and Weiss 1994). So herein, we developed a novel fluorescent method for rapid detection of the target DNA of genetic modified tomato based on the formation of DNA-Methylene Blue (MB) Complex (fluorescence probe) complex.

Materials and method

Reagents and solutions

All DNA sequences were synthesized by Shanghai Generay Biotech Co. (Shanghai,China). They were dissolved in a TE buffer (1 M Tris-HCl (pH 7.5) and 0.5 M EDTA) to make a 100 µM DNA stock solutions and stored at 4ºC until use. MB complex was kindly donated by Institute of Electrochemistry, University of Tehran, Tehran, Iran. The fluorescence emission spectra of MB were collected from 700 nm through a spectrofluorimeter (Varian,Eclipse) at room temperature. The excitation wavelength was set at

630nm. For the method calibration curve acquisition, F was plotted as our method signal, where F is the fluorescence intensity of the solution with addition of different concentration of target DNA. Analyses were always measured in triplicate, and the standard deviation was plotted as the error bar.

DNA extraction

DNA was extracted from transgenic plant tissue by using the NucleoSpin Plant II kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions with some modifications. Amount of 100 mg of transgenic tomato leaf powder were transferred to a sterile reaction tube followed by addition of 400µL of lysis buffer (PL1) and 10µL of RNase A. The mixture was mixed thoroughly and incubated at 65 ◦C for 10 min. The mixture was then centrifuged at 11,000×g in a microcentrifuge for 2 min for clarification of lysate. Three hundred microlitres of the supernatant were transferred to a NucleoSpin filter and centrifuged for 5 min at 11,000×g. Then, 450 µL of binding buffer (PC) solution were added and the solution was mixed and transferred to a NucleoSpin column to centrifuge at 11,000×g for 1 min. The column was washed three times with 400 µL of wash buffer (PW1) and centrifuged again at 11,000×g for 1 min. Then, 50 µL of elution buffer (PE) heated to 65 C and added to the column followed by incubation at 65 C for 5 min. DNA was eluted by spinning the column at 11,000×g for 1 min. After that, the purified DNA stored at 4 C. Finally 1 % Agarose gel electrophoresis of the DNA showed the integrity of the extracted DNA, while spectrophotometry gave the concentration and cleanliness.

PCR Analysis

Transgenic tomato plants contain cauliflower mosaic virus (CaMV) 35S promoter. So specific site of promoter gene was selected and then two primers designed to amplify DNA target sequence in this study. The 180 bp region of CaMV 35 S promoter was amplified using the designed primers Back: 5´-GCTCCTACAAATGCCATCA -3´ and Forward: 5´ -GATAGTGGGATTGTGCGTCA-3´. Each PCR mixture was prepared in a final volume of 50 µl containing 50 ng template DNA, 50 pM forward primer, 50 pM of the corresponding back ward primer, 40pM dNTPs, 25 mM MgCl2, 1.25 U Taq DNA polymerase (Fermentas) and 5 µl 10×PCR buffer II (Fermentas). Hot start PCR was performed at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and elongation at 72 °C for 1 min. The reaction was completed by a final extension time at 72 °C for 1 min. The amplified variable regions were purified by electrophoresis on a 1.5% agarose gel and subsequently extracted with an AccuePrep Gel Extraction Kit (Bioneer) according to the manufacturer's instructions. Finally the amplified target DNA denatured at 90 °C for 15 minutes to obtain target ssDNA.

DNA Hybridization and fluorescence detection

The 21 bp probe and two other mismatch and non complementary targets oligonucleotide used in this work were synthesized according to specific sequence of CaMV gene by Generay Biotech Co. The base sequence is shown as follows:

5'- GAACTTCCTTATATAGAGGAA-3'

5'- GAACTTCGTTATATAGAGGAA-3'

5'- AAGGAGATATATTCCTTCAAG-3'

Hybridization was carried out by gently stirring at 37°C. Basically, different concentration of target oligonucleotide was added to the 200 µL of the suspension of probe sequences in 0.01 M Tris-HCl and 0.20 M NaCl hybridization buffer and the mixture was incubated for 2 h by gently stirring in order to mixed thoroughly.

To prepare the sample solutions, 300 µL of MB (at 1×10^{-8} mol/L) was added to the mixture and incubated for another 50 min at room temperature. The fluorescent signals of the mixture were recorded with fluorescence spectrophotometer at room temperature.

Results and discussion

Fluorescence Influence of hybridized DNA on MB complex

The different responses in the fluorescence intensities of MB complex after hybridization were recorded with

three repetitive measurements. As the concentration of target ssDNA was increased, the fluorescence of MB complex gradually decreased (Fig. 1), which illustrated that the amount of DNA duplex at the sample increased through the hybridization process. The decreased values of fluorescence intensities of MB complex showed excellent correlation with the logarithmic values of ssDNA target and ranged from 3×10^{-9} to 1×10^{-7} M (inset in Fig. 1) with a regression equation of F= 2.70+4.39×108 c and a regression coefficient (r) of 0.992 with the detection limited of 2×10^{-9} M.

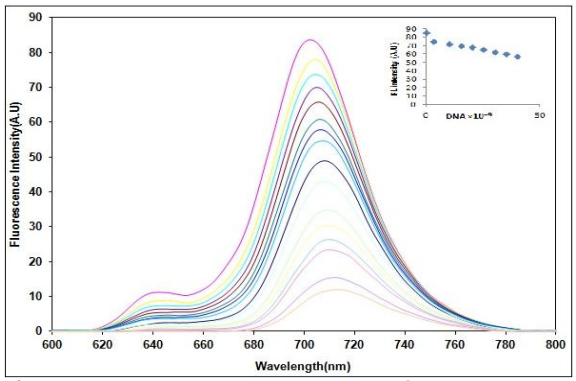

Fig. 1. Fluorecence spectra of 1×10^{-8} M MB in the presence of different concentration of target DNA, Inset: Logarithmic plot for fluorescence intensity ratio versus target DNA.

The obtained results showed that the fluorescence response of biosensor at a constant concentration of MB complex decreased by increasing the dsDNA concentration. It could be resulted from specific interaction of MB complex with the DNA structure which is imposed by the formed duplex.

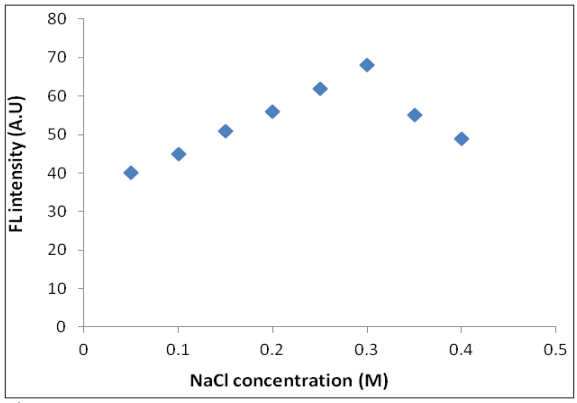

Fig. 2. Effects of different concentration of NaCl on MB fluorescent intensity.

Optimization of hybridization process

Presence of phosphate groups on the single stranded DNAs causes to dissociation of them in the solution and is robust hinder for duplex formation. So the presence of cations in solution could decrease this electrostatic repulsion and facilitate the hybridization process. So the influence of NaCl concentration on the hybridization were studied .As seen in Fig.2 the results showed that higher ionic strength through increase in NaCl concentration resulted to increase in fluorescence intensities of MB. But increase in concentration above 0.3 M showed small decrease in fluorescence intensities, so 0.3 M selected as an optimum NaCl concentration.

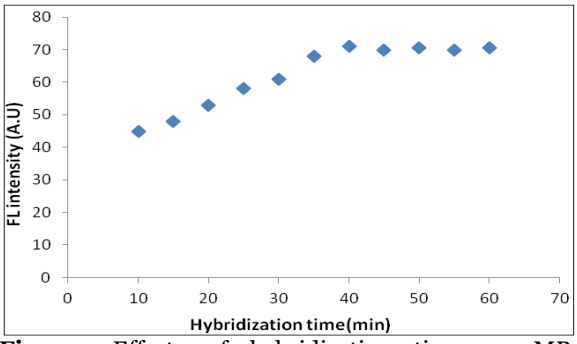

Fig. 3. Effects of hybridization time on MB fluorescence intensity.

The hybridization time as another assay condition on the experimental results was investigated. From Fig.3 it is clearly observed that increase in hybridization time from 10 to 40 min resulted to increase in fluorescence intensities and after 40 min it didn't change and remained constant .So the hybridization time determined in 40 min.

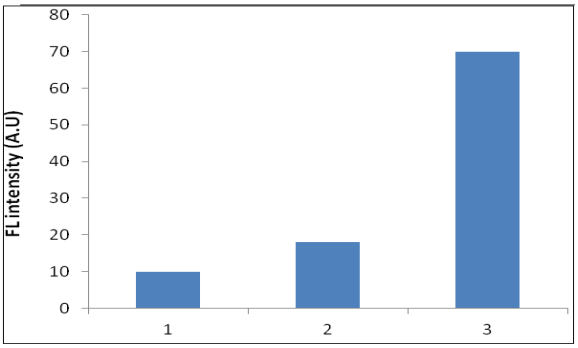

Fig. 4. Histogram of the fluorescence intensities for target DNA sequence a) uncomplimentary sequence, b) one-base mismatched sequence and c) full complementary sequence (concentration : 1×10^{-8} M)

Selectivity of detection method

The recognition of uncomplimentary and even single base mismatch is an important characteristic for a DNA biosensor. As it showed in Fig.4 the hybridization process of the DNA biosensor with complementary target, one base mismatch and uncomplimentary targets was investigated. It was found that there was no significant fluorescence decrease for uncomplimentary target while one base mismatch target showed a weak fluorescence quenching. This proved that when the hybridization process failed due to presence of uncomplimentary sequences there is no any interaction between MB complex and DNA resulted to lowest fluorescence intensity. Regarding to this assumption that full complementary dsDNA can bind to more MB complex it results to give the largest response than ssDNA.

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References

Ahmed MU, Saito M, Hossain MM, Rao SR, Furui S, Hino A, Takamura Y, Takagi M, Tamiya E. 2009. Electrochemical genosensor for the rapid detection of GMO using loop-mediated isothermal amplification. Analyst **134,** 966–972. http://dx.doi.org/10.1039/B812569D

Brodmann PD, Ilg EC, Berthoud H, Herrman A. 2002. Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. Journal of AOAC International **85,** 646–653.

Gao AG, Hakimi SM, Mittanck CA, Wu Y, Woerner H, Stark DH, Shah DH, Liang J, Tommens CM. 2000. Fungal pathogen protection in potato by expression of a plant defensin pepetide. Nature Biotechnology **18,** 1307–1310. http://dx.doi.org/10.1038/82436

Germini A, Mezzelani A, Lesignoli F, Corradini R, Marchelli R, Bordoni R, Consolandi C, Bellis GD. 2004. Detection of genetically modified soybean using peptide nucleic acids (PNAs) and microarray technology. Journal of Agricultural and Food Chemistry **52,** 4535–4540. http://dx.doi.org/10.1021/jf035355r

Huang HY, Pan TM. 2004. Detection of genetically modified maize MON810 and NK603 by multiplex and real-time polymerase chain reaction methods. Journal of Agricultural and Food Chemistry **52,** 3264–3268. http://dx.doi.org/10.1021/jf0499440

King CY, Weiss MA. 1994. The SRY HMG-Box recognizes DNA by partial intercalation in the minor groove: a topological mechanism of sequence specificity. Proceedings of the National Academy of Sciences of the United States of America **90**, 11990-11994.

Koziel MG, Beland GL, Bowman C, Carozzi NB, Crossland RL, Dawson J, Desai N, Wright M, Evola SV. 1993. Field performance of elite transgenic maize plants expressing an insectioncidal protein derived from Bacillus thuringiensis. Nature Biotechnology **11,** 194–200.

Lalonde S, Ehrhardt DW, Frommer WB. 2005. Shining light on signaling and metabolic networks by genetically encoded biosensors. Current Opinion in Plant Biololgy **8,** 574–581. http://dx.doi.org/10.1016/j.pbi.2005.09.015

Levandi T, Leon C, Kaljurand M, Garcia-Canas V, Cifuentes A. 2008. Capillary electrophoresis time-of-flight mass spectrometry for comparative metabolomics of transgenic versus conventional maize. Analytical Chemistry **80,** 6329–6335. http://dx.doi.org/10.1021/ac8006329

Qiu B, Zhang Y, Lin Y, Lu Y, Lin Z, Wong K, Chen G. 2013. A novel fluorescent biosensor for detection of target DNA fragment from the transgene cauliflower mosaic virus 35S promoter. Biosensors and Bioelectronics **41,** 168–171. http://dx.doi.org/10.1016/j.bios.2012.08.017

Xu WT, Huang KL, Wang Y, Zhang HX, Luo YB. 2006. A cotton-specific gene, stearoyl-ACP desaturase, used as a reference for qualitative and real time quantitative polymerase chain reaction detection of genetically modified organisms. Journal of the Science of Food and Agriculture **86,** 1103–1109.
http://dx.doi.org/10.1002/jsfa.2464