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Research Article

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Detection for Suspected Genetically Modified Maize and Soybean Crops in the Selected Places of Ethiopia

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Abstract

Detection of genetically modified organisms (GMOs) in crops is an important issue for all the subjects involved in seed quality control and customer's right. Due to the increasing number of GMOs research and development activity in the globe during the past few years, it has become necessary to screen and regulate highly practiced GMO crops. In this study soybean and maize crops samples were collected at different agricultural farm lands and commercial super markets that suspected for GMOs. Then, genomic DNA were extracted from crops samples by using Phenol chloroform method. By following ,the extracted genomic DNA amplified by using two oligonucleotide primer sequences such as CaMV35s and Lectin with the help of thermal cycler Polymerase Chain Reaction (PCR). In addition, positive control taxon -specific Invertase and Lectine genes for maize and soybean were used respectively. Finally, PCR amplicons were run on horizontal agarose gel electrophoresis for the confirmation of amplification. After that electrophoresis results were observed under gel documentation system. Invertase and Lectine gene events were detected in all maize and soybean samples, respectively. However, fortunately in all collected samples of maize and soybean in different places of the country the suspected foreign genes were not detected. Finally,this research result concludes that in the studied area soybean and maize crop samples are GMO free.

Keywords: GMO, Maize, Soybean, Transgene, CaMV35s Gene.

Introduction

Plants produced by transgenic technology or genetic engineering are called genetically modified organism (GMO).Now a day, there has been exponential growth in the field of modern biotechnology or genetic engineering for trait improvements starts from since 1996. In recent years surplus number of crop plant species have been genetically modified to attain traits such as biological defense against diseases and insects, thus reducing the need for chemical pesticides and introducing genetic traits that enable crops to better withstand drought, temprature,saline conditions and in addition, genetic modification has been used to improve various characteristics such as color, flavor, texture, nutrient value and shelf life of foods [1-7].

Many genetically modified crops have been approved worldwide since mid-1990s and the global area of these crops has expanded yearly since their commercialization. In 2011, 160 million hectares were cultivated with genetically modified crops mainly soybean, maize, cotton and canola [8]. A total of 26 countries, 19 developing and 7 industrial countries planted biotech crops in 2016. The top ten countries, each of which grew over 170million hectares in 2016, is led by the USA which grew 72.9 million hectares (39% of global total, similar to 2015), Brazil with 49.1 million hectares (27%), Argentina with 23.8 million hectares (13%), Canada with 11.1 million hectares (6%), India with 10.8 million hectares (6%), Paraguay with 3.6 million hectares (2%), Pakistan with 2.9 million hectares (2%), China with 2.8 million hectares (2%), South Africa with 2.7 million hectares (1%) and Uruguay with 1.3 million hectares (1%). An additional 16 countries grew a total of approximately 4.9 million hectares in 2016 [9].In 2019, the 24th year of commercialization of biotech crops, 190.4 million hectares of biotech crops were planted by up to 17 million farmers in 29 countries.

In Ethiopia Bt-Cotton is environmentally released since May 2018 after amendments of biosafety regulation in august 2015 to use GMO for commercial and research purpose [Ethiopian Biosafety regulation, 2015]. Other Africa country such as Burkine Faso,Sudan and Nigeria were allowed recently certain GMO crops for application. At the moment, several transgenes have been approved worldwide for cultivation and consumption as food and feed, while

consumers awareness are increasing regarding on food safety for more stringent regulations to control raw materials, import, export and distribution of GMOs [10, 11].

Therefore, the detection and identification of GMOs in food and seed have become important issues for all the subjects involved in food, import, and export seed control [12, 13]. Qualitative testing may be used to discriminate between the authorized and unauthorized GM food to identify safe or potentially unsafe material [14, 15]. Although, several analytical methods have been proposed, among them GMO detection methods involve for specific DNA sequence detection by means of PCR techniques. It is able to detect even small amounts of transgenes in raw materials and processed foods [16, 17]. Hence, in this study, we have examined the suspected recombinant DNA of genetically modified maize and soybean in different agriculture farming land and commercial areas of Ethiopia.

Materials and Methods

Maize and Soybean Crops Sample Collection

Suspected samples of maize and soybean crops for GMO were randomly collected at Commercial and Agriculture farming lands of different areas of Ethiopia. Those areas were Areba Minich, Gondar, Bahrdar, and Awash sebate, AddisAbaba, Awi Zone at Ayehu mechanized agriculture farming land and Chagni during the period September 2018-June 2019.

Genomic DNA Extraction of Soybean and Maize

Genomic DNA extraction was performed by transferring 1g powder of the grow ended soybean and maize by high-speed multi-functional crusher that can rotate 25000 RPM into a 50 ml falcon tube. In the flacon tube 30 ml CTAB Extraction buffer and 2.1 ml, 20% SDS were added. By following this, the mixture was incubated at 65°C for 30 minutes. To separate the supernatant from pellet the sample was Centrifuged for 20 minutes at 3,000 x g , then after the debris of the cell was existed in the pellet phase but DNA material was existed in the supernatant phase, therefore, 20 ml supernatant was transferred to a new 50 ml falcon tube using a 5 ml pipette. In the transfer DNA material 5 ml of 5M KAC was added and incubated on ice for 30 minutes . Then, it was centrifuged for 20 minutes at 3,000 x g and 20 ml supernatant was transferred to a new 50 ml falcon tube using a 25 ml pipette , also added equal volume of isopropanol by following this incubate on ice for 5 minutes and Centrifuged for 20 minutes at 3,000 x g.

Finally, the supernatant was discarded and the pellet was air-dried at 37°C until all isopropanol residues were evaporated then the pellet was completely dissolved in 10 ml TE for PCR reaction and stored at -20 °C for further use.

Determination of Purity of Extracted DNA:

The purity of extracted DNA were tested by 1.2 % Agarose gel electrophoresis.

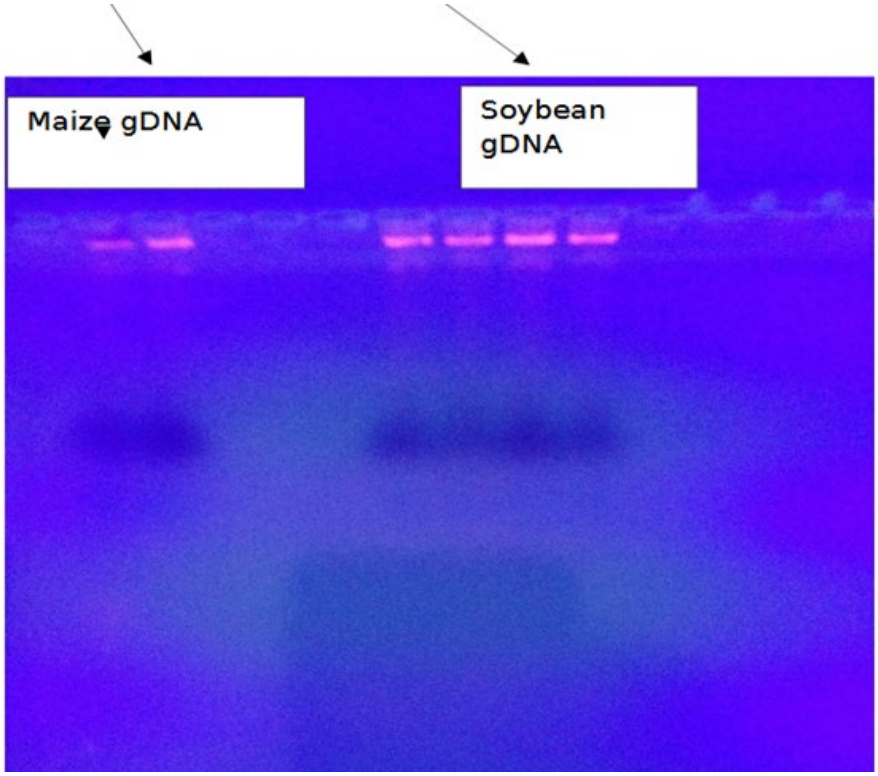

Figure 1: Extracted DNA from Maize and Soybean

DNA Amplifications

PCR reactions were carried out in a total volume of 20µL. Each reaction mixture contained 2 µL of 10x PCR buffer, 1.6 µL of 25 mM MgCl2, 0.2µL of Taq DNA polymerase, 1.6µLof 2.5 mM dNTPs, 4µL of extracted DNA, 2µL of 10 µM for all foreword and reverse Primer then finally 6.6 µL nuclease-free water were added to the reaction to detect the expected gene from DNA of maize and soybean.

Thermal cycler reaction conditions were for pre-incubation at 95°C for 5min,40 cycles consisting of dsDNA denaturation at 95°C for 20 sec, primer annealing for 40 second at 57°C ,for extension at 72°C for 60second and final elongation at 72°C for 3min.

The amplified PCR products of 5 µl of each and 3µl loading dye were electrophoresed into 2 % agarose gel in 1x TBE buffer, stained with5 µl ethidium bromide and visualized by gel documentation system with ultraviolet light and the amplification products were determined by using 3µl of 100 bp DNA marker

Table 1: Primers were used for GMO Detection and its Sequence

Primers Name	Gene sequence	Expected amplified gene
35S Promoter	*F-5-GCTCCTACAAATGCCATCA-3*	CaMV gene
	R-5-GATAGTGGGATTGTG CGTCA-3	
Lectine	*F-5-GCCCTCTACTCCACCCCCATCC -3*	*Lectine* gene
	R -5-GCCCATCTGCAAGCCTTTTTGTG -3	
Invertase	*F-5-CCGCTGTATCACAAGGGCTGGTACC -3*	Invertase gene
	R 5- GGAGCCCGTGTAGAGCATGACGATC-3	

Result

Normal thermal cycler PCR-based method were used to detect the suspected crops for genetically modified maize and soyabean. PCR reactions were performed by using a 35S promoter-specific primer and the target plant host gene primer for Invertase and Lectine gene as positive control . The PCR products were analyzed in electrophorosis via 2% agarose gel.

The primer pair Lectine, specific for the single copy endogenous lectin gene in the collected samples of soybean were detected and its PCR product were 118 bp size as mentioned earlier [18]. The other primer pair Invetrase, specific for the endogenous gene Invertase was amplified to give a 226 bp amplicon [19]. This product was also detected in all collected sampls of maize crops. However, the desirable PCR products of 195 bp fragment of CaMV 35S promoter was not amplified in soybean and maize.

Therefore, these primers can be used for the routine screening and detection of specific gene in suspected GM soybean and maize. Most of the new analytical methods used for detection and quantification of transgene in GM plants depend upon real time PCR assays, DNA-Chip technologies and using GMO screening kit, which are very expensive. However, the inexpensive thermal cycler and gene specific primer method used in the present study would be more suitable for meeting regulatory obligations in developing countries.

One hybrids of GM cotton were approved in Ethiopia for commercialization in May 2018. However, the detection methods on different levels, such as qualitative, quantitative, national or international validated and non-validated, have been not developed to check really the commercialized Cotton is GMO or not, these detection methods and monitoring programmers of GM food/derivatives, import and export crops were not established in Ethiopia for a long time. However, time has come now to put in place the detection procedures, protocols and established a laboratory for GM planting material in different crops by Ethiopian Environment and Forest Research Institute.

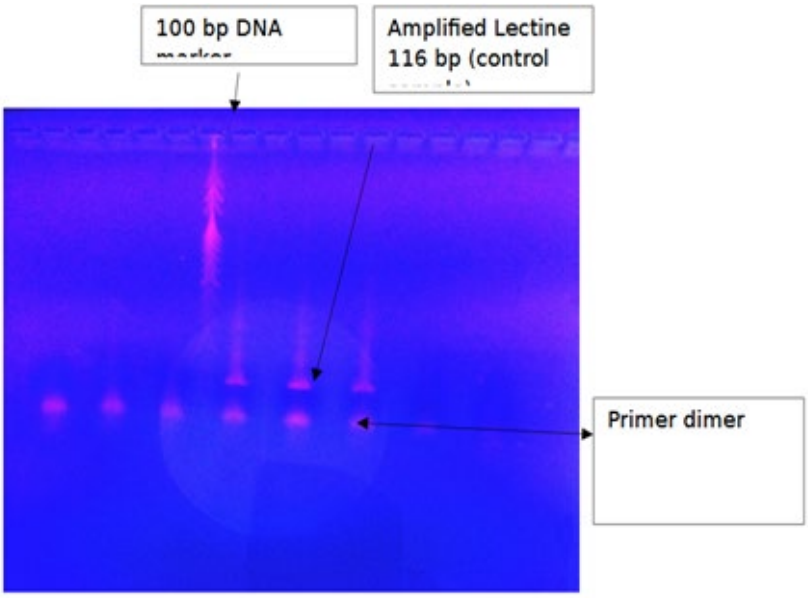

Figure 2: Amplified PCR products of soybean

Table 1: Areas of Analyzed Maize and Soybean Crops

Crops	Sample collected area	No of test product	No.Detected GM products
Maize	Awashe sebate	1	no
	Ayehu Mechanized farm	1	no
	Arba -Minch	1	no
	Chagni	1	no
Soybean	AddisAbaba	1	no
	Ayehu - Mechanized farm	1	no
	Gondar	1	no
	Bahrdar	1	no
	Chagni	1	no

GM indicates genetically modified products deteced in this study

Discussion

Present results showed that transgenic maize and soybean crops are not cultivated in the studied areas of Ethiopia. These were identified by specific primers 35S and specific for endgnous genes of maize and soybean. The amplicons of the specific sequences detected are used to build the different GMOs and regulate expression of transgenes, such as promoter 35S and the terminator NOS [20]. Furthermore, PCR is a useful machine in detecting the transgenic plants [21].

From the time when first generation of GMOs seed were released for plantation in 1995 and their products entered the food and feed markets of various countries in 1996 there have been reports questioning the safety of these organisms and their products when used as food or feed [22]. This brought about an active debate and controversy on possible risks that might be caused by such crops and their products to health and environment [23, 24]. As a result two main methods for the identification of GM food and feed have been reported in the literatures; PCR and Enzyme-Linked Immunosorbent Assay (ELISA). PCR is the most accepted technique used worldwide; it showed consistent results when using specific primers for the detection of the regulatory sequence or structural gene in the inserted gene fragment [25]. The designed primers must possess some specific characteristics and can be used for GM product screening and product-specificity detection. Thus, it might be convenient to consider these methods for the detection and identification of GMOs. This would represent a new field of diagnostics in which a great deal of development has already been accomplished.

Conclusion

Finally, the soybean and maize has been tested for the presence or absence transgene using thermal cycler PCR, fortunately there is no GMO crops in the studied area. The protocol developed can be

used to meet the regulatory obligations and for the labeling of GM plants and their derivatives for future in Ethiopia. The PCR method described in the study is an efficient and sensitive tool, which can be applied for routine screening/testing of herbicide resistant GM soybean and GM maize from conventional/non-GM soybean or maize.

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