

# SCREENING FOR GENETICALLY MODIFIED ORGANISMS SEQUENCES IN FOOD SAMPLES IN BOTSWANA USING THE BIOSMART ALLIN 2.0 GMO SCREENING SYSTEM

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## ABSTRACT

*We used the Allin 2.0 GMO screening system from Biosmart, Switzerland to screen for the presence of genetically modified food sequences in maize meal samples, fresh fruit and vegetables from some retailers around Gaborone, Botswana. The Allin 2.0 is a multiplex PCR system for the detection of genetically modified organisms that contain the 35S-promoter and/or the NOS-terminator derived from the cauliflower mosaic virus and the Agrobacterium tumefaciens, commonly utilized in commercially grown transgenic crops. From the food commodities analyzed, two brands of maize meal were found to contain a 227 bp fragment amplified by the 35S promoter, which indicates the presence of genetically modified sequences within the products.*

**KEY WORDS:** *Genetically modified organisms (GMOs), 35S-promoter, nopaline synthase (NOS) terminator, PCR, Botswana.*

## INTRODUCTION

There has been concern in the recent past by the general public about the consumption of genetically modified foods (GM foods) and this has been emphasized by Bennett *et al.*, [1]. This has become an international debate with one group lobbying for the consumption of such foods and the other being against on the grounds that there may be dangers and risks associated with the consumption of such foods. Suggestions towards labelling of such foods so that the public can be informed of what they are consuming have been put forth [2,3,4], and the

widespread demand for labeling of foodstuffs containing genetically modified organisms (GMOs) has created a need for accurate GMO testing methods [5, 6]. Currently, the labeling of transgenic foodstuffs is required in some countries such as the European Union, Australia, New Zealand and Japan, while other countries including the United States, Canada and Botswana have not yet adopted requirements for GMO labeling. Botswana has no legislation in place to govern and regulate the use of GM foods and the Department of Agricultural Research at the Ministry of Agriculture [7] is currently working on a National Biosafety Framework

(NBF). The NBF is a combination of policy, legal, administrative and technical instruments that are set to address safety for the environment and human health in relation to modern biotechnology. It is also aimed at creating awareness on the development of appropriate legislation concerning GMOs in Botswana.

Botswana imports most of her food supplies from neighbouring South Africa and yet South Africa produces cereals, fruits, vegetables and ornamentals through genetic modification [9]. Cereals that have been produced by genetic modification include maize, wheat, barley, sorghum, millet, soybean, lupins, sunflower and sugarcane. Some of the fruits that are produced by genetic modification include apricot, strawberry, peach, apple, table grapes and banana. Most of the crops derived from modern biotechnology consist of varieties that have been modified through the introduction of one or more genes (transgenes) that code for insect or disease resistance, herbicide tolerance, or a combination of these traits [10].

Transgenic plants usually contain three essential elements: a transgene, a promoter and a terminator. Although many potential promoter and terminator elements have been identified, the most commonly used are the CaMV 35S promoter, from the 35S RNA of the phytopathogenic cauliflower mosaic Caulimovirus, and the NOS terminator, derived from the nopaline synthase gene from the *Agrobacterium tumefaciens* Ti plasmid. Almost all commercially developed transgenic crop plants contain either the CaMV 35S promoter or

the NOS terminator. For example, the genetic modification of a commercially available soybean involves the introduction of a glyphosate-resistant 5-enol-pyruvyl-shikimate-phosphate synthase (EPSPS) gene and the elements used for the EPSPS gene expression are the cauliflower mosaic virus (CaMV)-derived 35S-promoter and the nopaline synthase (NOS) transcription terminator sequence [11, 12]. The Allin 2.0 GMO screening system from Biosmart, Switzerland is a multiplex PCR system for detection of GMOs that contain the 35S-promoter (35S) and/or the NOS-terminator derived from the cauliflower mosaic virus and the *Agrobacterium tumefaciens*, as well as internal control sequences. The 35S and the NOS sequences are the most common promoter and terminator, respectively, utilized in commercially grown transgenic crops. In addition to transgenic markers, this multiplex system contains soya (lectin) and maize (zein) specific primers used to demonstrate the presence of these two species in a sample. An internal control determines inhibitors in the reaction. The internal control DNA utilizes the same primer binding sequences as lectin and will cause competition.

The ability to detect these elements will allow detection of the vast majority of GMO plant material as these genetic elements do not naturally occur in agricultural crop sources. This study therefore looked at the use of the Allin 2.0 GMO screening system and a simplified DNA isolation protocol to screen for the presence of GMOs in some food items including maize meal,

fresh fruit and vegetables from some retailers

## **MATERIALS AND METHODS**

### **a. Screening kit**

The Allin 2.0 was used to screen for the presence of GMOs in maize meal, fresh fruit (pear and mango) and vegetables (carrot and tomato) from some retailers around Gaborone, Botswana according to the suppliers protocol [13].

### **b. DNA extraction from maize meal, fresh fruit and vegetables**

A common method to determine whether foodstuffs contain genetically modified ingredients involves purification of genomic DNA from the material in question and subsequent analysis by PCR amplification. Maize meal samples, fresh fruit and vegetable material were purchased from local retailers around Gaborone. Samples were homogenized by grinding in a pre-cooled mortar with a pestle in the presence of polyvinyl (poly) pyrrolidone, (PVP) according to a modified method by Murray and Thompson, [14]. One gram of (fresh weight) tissue was flash frozen in a precooled motor in liquid nitrogen and 500 mg of PVP was added and the material was ground to a fine powder with a pestle. The powder was transferred to a 2 ml eppendorf tube and 1 ml of hot extraction buffer [2% CTAB (w/v), 1.4 M NaCl, 0.2%  $\beta$ -mercaptoethanol, 20 mM EDTA, 100 mM Tris-Cl (pH8.0)] (60°C) was added. The tubes were incubated in a hot water bath (55-60°C) with occasional mixing for 30 – 45

around Gaborone, Botswana.

minutes. An equal volume of chloroform [chloroform:iso-amyl alcohol (24:1)] was added and the mixture was vortexed briefly. The tubes were then centrifuged on a refrigerated bench top centrifuge (Eppendorf centrifuge 5415R) for 10 minutes at 10 000 rpm. The supernatant was transferred to a fresh eppendorf tube, and one or two more chloroform extractions were performed until supernatant was clear. One tenth volume of 5 M ammonium acetate was added to the supernatant, followed by 0.54 volumes of cold propan-2-ol. This was left at -20°C for 10 minutes to precipitate the DNA. The DNA was pelleted by centrifuging at 14 000 rpm for 10 minutes. The supernatant was removed and the DNA pellet was washed in 80% ethanol. All the ethanol was removed and the pellet was dried in an eppendorf speed-vacuum drier (Eppendorf Concentrator 5301) and resuspended in 30  $\mu$ l TE buffer [TE = 10 mM Tris.Cl, 1mM EDTA, pH8.0]. Some of the samples were treated with RNase A during extraction, some were resuspended in buffer containing RNase A and others were not treated with RNase at all (see results and discussion). The DNA concentration was estimated using ethidium bromide fluorescence against known lambda DNA standards on 0.8% agarose gels.

### **c. Amplification reaction**

The DNA template was diluted to 16ng/ $\mu$ l in distilled water. Each polymerase chain reaction mixture consisted of 29.8  $\mu$ l of the MasterMix Allin 2.0, 5  $\mu$ l 10X PCR buffer (Promega, USA),

0.2 µl GoTaq®Flexi DNA polymerase (5U/µl) (Promega, USA), 5 µl of the internal control and 10 µl of 16 ng/µl template DNA. The micro centrifuge was flicked then centrifuged briefly to mix the contents before insertion in a thermal cycler (Techgene thermocycler (Techne)). DNA samples were amplified using a touch down PCR profile using the following amplification programme. Pre-denaturation, 15min/95°C, followed by denaturation, 20sec/95°C, annealing, 60sec/66°C for 5 cycles; denaturation, 20sec/95°C, annealing, 60sec/62°C, extension, 60sec/72°C for 5 cycles; denaturation,

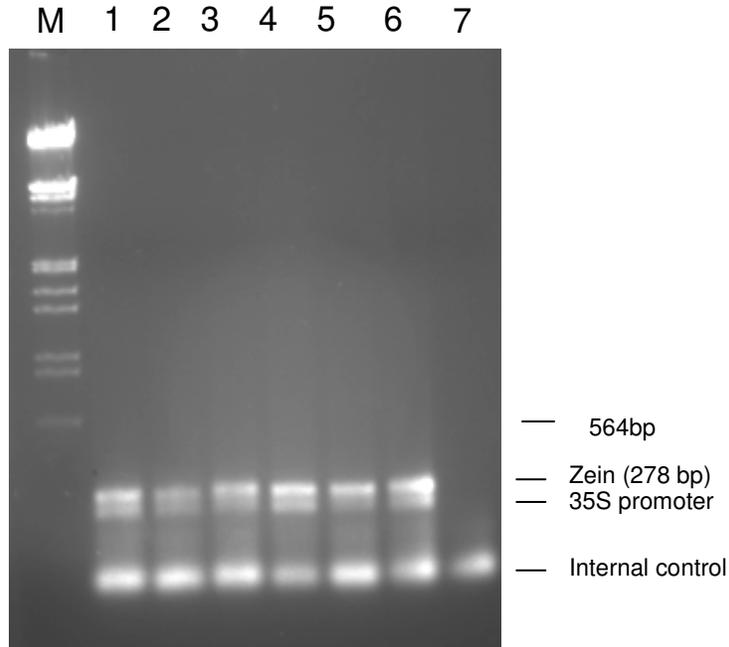
20sec/95°C, annealing, 60sec/58°C, extension, 60sec/72°C for 5 cycles; denaturation, 20sec/95°C, annealing, 60sec/54°C, extension, 60sec/72°C for 10 cycles; denaturation, 20sec/95°C, annealing, 60sec/50°C, extension, 60sec/72°C for 15 cycles and finally denaturation, 20sec/95°C, annealing, 60sec/45°C, extension, 20sec/72°C for 15 cycles. At the end of PCR, 20 µl aliquots of the PCR products were electrophoresed on 1% agarose gels containing ethidium bromide in 1 x TAE buffer at 80 volts for 1 hour and viewed on the Gene/Genius Bio Imaging System (Syngene).

## RESULTS AND DISCUSSION

The concentrations of the various DNA samples were determined using agarose gel fluorescence against known lambda DNA standards as the sample concentrations could not be determined spectrophotometrically due to the presence of RNA in some of the samples. The procedure and the cycling parameters used in this study were those recommended by Biosmart [13].

A 278 bp zein (maize) fragment and a 227 bp 35S promoter were amplified from the maize meal sample, together with the 132 bp lectin fragment from soya that serves as an internal control (Figure 1). The 227 bp fragment indicates the presence of the transgenic 35S

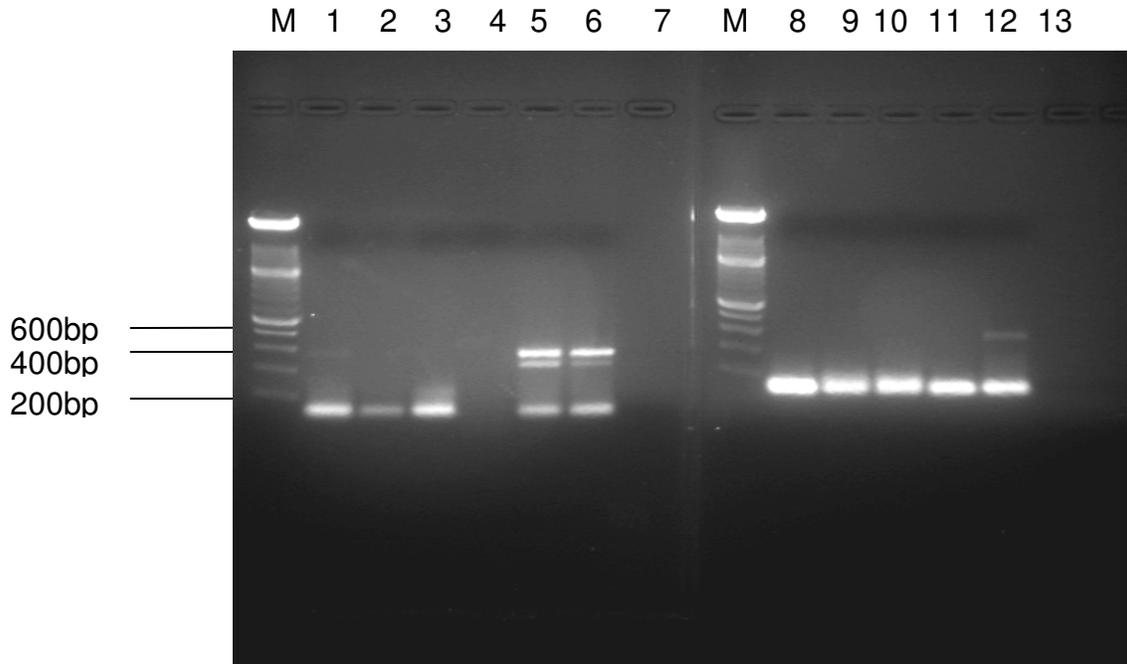
promoter sequence in the maize meal samples used in this study compared to the negative control (Figure 1, lane 7) that did not display the fragment. The same samples were subjected to different treatments with or without the use of RNase A (Figure 1). Samples in lanes 1 and 5 were not treated with RNase A, while those in lanes 2 and 4 had RNase A added during the extraction procedure and samples in lanes 3 and 6 were resuspended in TE buffer containing RNase A. Even though the suppliers of the Biosmart Allin 2.0 kit emphasized that samples be treated with RNase A, the presence of RNA was found not to have any observable inhibitory effect on the amplification reaction (Figures 1 and 2).



**Figure 1. PCR amplification products from maize meal samples.** A 20  $\mu$ l sample from each amplification was subjected to electrophoresis on a 1% agarose 1X TAE gel. Lane M is the  $\lambda$ -DNA- *Hind* III/*Eco*R I digest molecular size marker. Lanes 1-3 are Fresh Mill Super Maize meal, Lanes 4-6 are A1 Maize meal and Lane 7 is the negative control (water).

Normally the DNA isolation kits supplied by most commercial suppliers are costly and it becomes a deterrent for a number of laboratories in third world countries to test for GMOs mainly due to the cost element [15, 16]. We report here the development of a simplified protocol that enabled testing for the presence of GMOs in various fresh samples including fruit, and vegetables. This procedure is an efficient process for isolation of DNA that is free of inhibitors (as evidenced by the amplification of

the internal control) suitable for amplification from a variety of sources. When a soya sample was amplified, clear competition was observed in the 132 bp lectin fragment in the internal control which was out competed by the 163 bp lectin fragment in the sample being analyzed (results not shown). As reported by the suppliers, the internal control DNA uses the same primer binding sequences as those for soya lectin and will cause competition.



**Figure 2. PCR amplification products from maize meal samples, fresh fruit and vegetables.** Lane M is the 100 bp ladder, lane 1 and 2 are 3% GM maize, and lane 3 is 3% GM soya, used as positive controls. Lanes 5 and 6 are Fresh Mill Super Maize Meal and A1 maize meal respectively. Lane 8 = pear, lane 9 = carrot, lane 10 = tomato, lane 11 = mango, and lane 12 is positive control. Lanes 4, 7 and 13 are blank.

This is the first known attempt at testing for the presence of genetically modified sequences in fresh fruit and vegetable samples in the Botswana market and even though the study points to the presence of GM sequences in processed foods, further analysis is required to optimize the procedures for fresh fruit and vegetables. The amplification of the internal control sequences in the various fruit and vegetable samples in Figure 2 (Lanes 8-11) indicates that the DNA isolated by the CTAB

method is free of inhibitors, and based on the results, we can say that the fruits and vegetables analyzed are free from GMO sequences. Further analysis of the samples must be conducted and even though the CTAB method is laborious, it needs to be explored further in such analysis given the cost implications with kits. Another alternative could be to explore individual primers as the nested PCR was optimized for processed food and might not necessarily work for fresh samples.

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