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Rapid and Efficient Detection of Genetically Modified Organisms (GMO) in Flour

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Abstract: **Routine tests for genetically modified organisms (GMO) detection in flour samples are widely developed, these led to the apparition of multiple methods based onto the deoxyribonucleic acid (DNA) extraction rather than the efficient techniques available. The aim of this study was to investigate flour content. Flour samples from known and unknown sources were assayed according to a modified protocol of DNA extraction. Whether the material sources were powder, the protocol we developed was based onto the adaptation of known protocol of DNA extraction from leaves to a suitable technique adapted to extract a high quality of genomic DNA from flour. The consequent accurate investigation previously needed a strict control of DNA that was successfully used as template. Polymerase chain reaction (PCR) analyses permitted to demonstrate the biological sources of the flour and notably the stable integration of the following foreign genes in maize. Identification of GMO through the DNA extraction method routinely used in leaf tissue widely demonstrated the efficacy of this strategy in flour.**

Keywords: **Flour, GMO Detection, Corn, DNA, PCR.**

I. INTRODUCTION

Until about 20 years ago the investigation of food containing GMO products was regarded as a safety concern. Since then, the developments of technologies for GMO detection were rising. While the detection of GMO in foods is presently regarded as either a quality or safety criteria for consumers many laboratories were involved in the development of their own techniques for tracing GMO food. Traceability of GMO is presently a big concern of any worldwide government for pointing out the high quality of food issues and notably their status of GMO-free.

Detection of biological molecules follows the logical sources of these different foods. Referring to the molecular biological dogma that any foreign gene is engineered in plants according to the *Agrobacterium tumefaciens* system, DNA-based technologies are the first and well known techniques employed. For this, development of PCR techniques targeting the genomic plant DNA is the wide-spread approach [1]. To evaluate the presence of GMO food products in a processed matrix (liquid or solid), the extraction methods of either DNA, RNA or proteins require a suitable protocol validating the planned assays. Expectedly a processed food matrix is not often pure, e.g. flour can contain fiber, starch and unknown chemical residues (polysaccharides or polyphenols) [2]. While DNA extraction techniques were referred to the heat of flour sample that was consequently diluted in an appropriate solvent, spectrophotometry and agarose gel electrophoresis AGE were only the techniques that permitted to conclude about their appropriateness. In developed countries, high technologies about GMO detection are available [3]. While big Research and Development consortium was created throughout the world for tracking GMO [4]. The major approach of these techniques was either the protein derived transgene product or the targeted nucleic acids. Exploration of genomic DNA was the most targets utilized. Detection of foreign DNA remains the most templates issuing of the particular interest of regulators. Within the help of researchers and the progress achieved in molecular analyses, GMO detection becomes a reality. Presently, it is easier to trace GMO products in any food and quantitatively confirm their presence.

The main objective of this work was to detect foreign genes engineered in flour. Using maize and wheat commercial flour as control, we were able to screen corn and wheat flour, detect CaMV (cauliflower mosaic virus) promoter, NOS (nopaline synthase) terminater and glyphosate transgenes in corn by using different primers. These results show that the housing methods for DNA extraction from any flour are really appropriate for GMO detection.

II. RESULTS AND DISCUSSION

A. Appropriate template DNA:

To achieve the findings of foreign gene in flour, DNA extraction was the basic step for getting genomic template appropriate to the targeted detection. Two methods were assayed, the first was based onto the available kit (Macheney-Nagel GmbH, Düren, Germany) and the second was an adapted protocol routinely employed in woody plant DNA extraction. The CTAB (Cetyltrimethylammonium bromide) extraction method was adapted for genomic studies. Spectrophotometry data (not shown) pointed out that both types of genomic DNA are apparently equal. The choice of DNA extraction method is crucial because Taq DNA polymerase requires clean reagent mixture that will not interfere with undesired substances challenging the production of targeted amplicons [5-6]. While the literature evoked the problems within DNA protocols requiring organic solvents [7], several

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methods pointed out the availability of commercial kits. Trying to extract DNA of the flour materials with the Macheney Nagel DNA kit, that was available at the laboratory, the amount of DNA obtained was low. While the quality looks to be good, at the end of the analysis, the extracted DNA was not appropriate to PCR analysis because no band was detected with the primer pairs to explore either the 5S ribosomal DNA or the zein gene (not shown). Regardless to the leaf or flour material sources we concluded that the soil DNA kit was not suitable. An intermediate protocol including flour heating and routine leaf protocol of Kobayashi *et al.* [8] within CTAB permitted to extract the same quality of DNA. Moreover the amount of collected DNA was higher and appropriate for PCR analysis. Figure 1 shows the similarity of the extracted materials, there are no smears appearing in each lane. The comparative studies for plant gene detection showed that the modified method of leaf DNA extraction is more relevant and confirmed the inappropriateness of a kit excluding CTAB. However the amplicon of 5S ribosomal DNA showed that each flour came from plant source (Figure 2). These data confirm the integrity of DNA extracted from the three unknown flour, the commercial wheat flour and obviously from corn leaves.

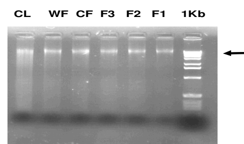

Figure 1. Gel electrophoresis showing the 1Kb ladder (Invitrogen, Life technologies, USA) and the genomic DNA extracted from five different flour: the three unknown (F1, F2, F3), the two other commercial from corn (CF) and wheat (WF) flour and corn leaves (CL).

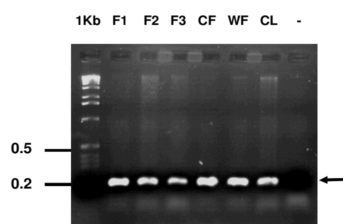

Figure 2. Gel electrophoresis showing the amplicon resulting from the PCR using the primer pair targeting the 5S plant ribosomal DNA. Lane (-) represents the control without DNA template.

B. Corn flour identified via *PCR:*

We recognized that we do not have ample information about these flour samples so we need to develop strategy to identify the sources of these materials and then target their content. Our study gave us a clue about how to identify the sources of flour. Using DNA analysis to provide the most significant approach, we then address this concern about molecular tools that allow to develop a rapid and accurate technique to investigate these flour samples.

Figures 2 and 3 confirm that the Taq DNA polymerase works. PCR amplification with the specific primers of zein gene resulted in a band of 500 bp in the commercial flour and leaf corn extracts. Interestingly Figure 2 also shows that the zein gene is also detected from DNA extracted of the three unknown flour samples (F1-3). That points out that all three different flour came from corn, however the DNA extracted from wheat flour does not contain this gene. These assays were useful both to verify the integrity of the DNA extracted by short baking with CTAB and identify the biological sources of three different flour. The lack of cross reaction with the commercial wheat flour confirms such observations. These studies demonstrate that PCR efficiency requires clean DNA, appropriate for any molecular testing.

- CL WF CF F3 F2 F1 1Kb

0.5

Figure 3. Gel electrophoresis showing the amplicon resulting from the PCR using the primer couple targeting the zein gene

C. GMO events:

While a range of technologies based onto molecular biology techniques can be used to detect GMO events in flour. In this regard, strategies to analyse flour DNA were rationally build in order to gradually identify their content. To detect the nature of engineered transgene in the three respective flours (F1, F2 and F3), PCR studies were carried out with different primer couples (Table 1). While a lot of GM crops were produced in different laboratories across the world, only a few were approved and presently released [10]. In the previous item we strongly identified the biological sources of these flour as maize, the expanded analyses were then directed to the detection of MON810 and NK603. Both GM corn have common foreign genes (NPTII marker gene) and basic either promoter (CaMV 35S) or terminater (NOS) sequences [13]. So attempts to detect CaMV35S and NOS sequences

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in relation to GMO event were chosen. Using two respective couple of primers that we designated (Table 1), the predicted challenging studies reveal how 35S and NOS were present in flour F2 and F3 (Figures 4 and 5).

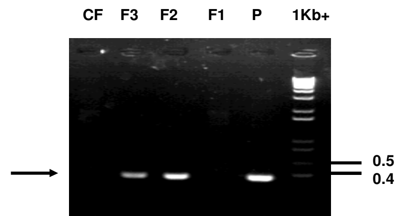

Figure 4. Gel electrophoresis revealing the amplicon from the PCR reaction using the primer couple targeting the CaMV35S promoter. Lane P represents the positive control pBI121 plasmid harbouring the CaMV35S promoter.

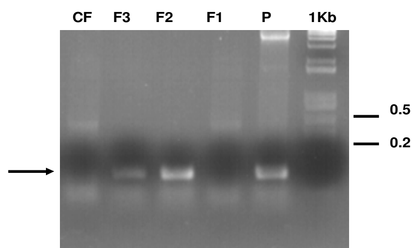

Figure 5. Gel electrophoresis showing the amplicon from the PCR reaction using the primer pair targeting the NOS terminater sequence. Lane P represents the positive control pBI121 plasmid (Jefferson et al, 1987) harbouring the NOS terminater

As Figures 4 and 5 showed, flour F2 and F3 were qualified as GMO food. To identify the GMO event possibly occurring in these two respective flour, two trials of PCR studies were set up, the one was to target the MON810 event and the second to the NK603 event. The detection studies to which the respective ESPSP4 (phosphoskimate) gene confering the herbicide glyphosinate resistance gene (NK603) and the c*ryIA(b)* (*Bacillus thurigiensis*) gene leading to the GM corn MON810 to harbour a pesticide gene were also performed. Referring to the specific primers published in the literature [13], these two latter sequences can be respectively detected through PCR. Figures 3 to 5 show the different content of the three analysed flour.

Referring to the conducted studies with the specific primers targeting the glyphosate transgenes, we can exhibit the originality of flour F2 and F3 in Figures 6 and 7. Flour F2 derived from corn MON810 and the F3 flour harboured both MON810 and NK603. Unless stated the case of the flour F3, the data obtained did not permit to qualify whether that flour resulted from the cross contamination of the two F2 and F3 flour or simply a mixed flour! Excluding the possible source of this third flour from a hybrid GM corn deriving from both MON810 and NK603 event, our rationale is following the ethical respect of GM technology. A foreign gene is introduced in a bait crop for a specific and targeted issue to improve GM corn production in an environment either infested with pest or largely occupied by weeds. When comparing two parallel studies aiming at the identification of NK603 and MON810 event, the PCR study designated by Hsin-Ying and Tzu-Ming [13] allowed to point out that MON810 event is positive for both. However NK 603 event is only contained by flour F3.

In respect to the international consideration about bioethics, the use of products deriving from GM technology should be traced [4]. According to these studies, our aim was to respond to the quested source of food materials. The technology developed here was basic but sufficient enough to follow up the origin and sources of food materials. Regardless of the form of the derived GMO products (processed flour from grain) that can be imported in any foreign countries, obviously there is some diversity between industrial and developed countries but the efficiency of the detection techniques points out that the processed flour is similar. Validation studies carried on these flours showed the detection of GMO events in all three flours. Although such analytical studies in an industrial country allowed both to determine the biological sources of these flour and notably the accurate amount of GMO traces [9], the GMO detection in developed country restrictively remains analytical. Does such food already consumed by millions of human for years in several developed countries require some particular labelling [4-10].

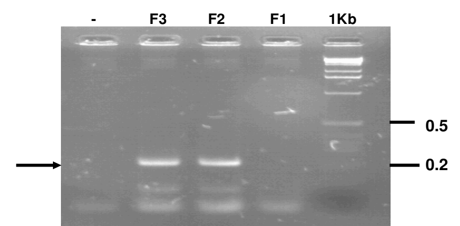

Figure 6. Gel electrophoresis showing the amplicon resulting from the PCR using the primer couple targeting the MON810 event.

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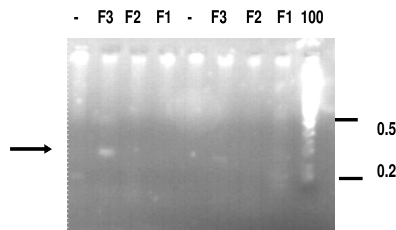

Figure 7. Gel electrophoresis showing the 100 bp ladder and the amplicon resulting from the PCR using the primer pair targeting the NK603 event.

III. Experimental Section

A. *Flour samples:*

Three sources of unknown flour suspected to contain GMO events were spread by SANGL (Southern African Network for GM Detection Laboratories) institute to different African laboratories. To examine their respective nature and source, three other DNA sources, two first from commercial flour (corn and wheat) and the third from maize (*Zea mays*) leaves, were included in the analyses. Regardless of their origin, flour was apparently similar and no extra attention was paid to the respective granulometry of the five flour samples.

B. *DNA extraction and reagents:*

As developed by Balasubramanian *et al.* [11], flour samples could be ground in an appropriate buffer with or without organic solvent. While the DNA kit from Macheney Nagel (GmbH, Düren, Germany) was initially used, we also assayed the "housing" method wherever the DNA was extracted from the combined heated flour as reported by Balasubramanian *et al.* [11], and that routinely applied to extract genomic DNA from leaves [8]. While the first step including leaf grinding and fast spinning at 12K was avoided, because flour was directly suspended in Tris-NaCl-EDTA-SDS buffer and heated at 65 °C as performed by Balasubramanian *et al.* [11]. To avoid the statistical degradation of genomic DNA contained in an unknown matrix possibly harbouring fiber, polysaccharides and polyphenols, a modified protocol was set up by short baking of the mixture for 15 min. The second step within Tris-EDTA-Sorbitol-SDS TESS buffer was similarly processed with the routine leaf treatment. While the supernatant was a bit viscous, this was reduced with the isopropanol precipitation of DNA. The DNA concentration solutions was then measured through spectrophotometry and the quality appreciated within the 260/280 nm ultraviolet absorption ratios [12].

C. *PCR analysis and GMO events:*

Two major GM maize crops were approved by the European commission (EP1582592B1) and spread across the world. The integration of the CP4 EPSPS gene into corn genome produces a foreign protein to confer tolerance to glyphosate. Such corn designated as NK603 is widely exploited in field and nutritionally used for flour. In parallel the corn event MON810 is linked with the introduction of the c*ryIA(b)* (*Bacillus thurigiensis*) gene leading to the GM corn MON810 to harbour a foreign gene conferring resistance to pest. Both foreign genes could be targeted through PCR and their absence in commercial control flour confirmed the GMO trait identified from these flours. Different primers designated by Hsin-Ying and Tzu-Ming [13] to probe the glyphosate transgenes (NK603 and MON810) and those targeting the CaMV (cauliflower mosaic virus) promoter and NOS (nopaline synthase) terminater were synthesized to detect the GMO events possibly occurred in flour samples. To determine the nature of the crop source, specific primers matching to the zein gene basically present in maize were utilized [13]. PCR analyses were performed with Taq DNA polymerase in 25 µL of Qiagen reagent (Qiagen, Valencia, CA, USA) according to different conditions (Table I and II). PCR amplification was carried out using an Eppendorf Mastercycler-Pro thermal cycler (Eppendorf, AG, Hamburg, Germany). The amplified bands were loaded onto an agarose gel and fractionated through electrophoresis. Ultraviolet light observations permitted to visualize the amplified DNA.

IV. Conclusion

Here we present a new and simpler approach to perform studies for rapid and accurate detection of GMO in flour using PCR technologies. Taking advantages offered by the PCR analysis, the present protocol of DNA extraction helped minimize time. In this regard, we validated a model identification technique that is readily suitable for laboratory in a developed country. The importance of partnerships between local and EU laboratories is supportive. This will make easy the building of relevant laboratory assisted by human and financial capital answering to the societal demand in a sustainable manner.

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Conflict of Interest

The authors declare no conflict of interest.

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Table I. List of the different primer pairs and the expected amplicon targeted

Sequences	Target	Amplicon (bp)
Forward ctctcggcaacggatatctcggctctc	5S ribosome	216
Reverse gggggcaacggcgtgtgacgccca	5S ribosome	
Forward cctcagtcgcacatatctactatact	Zein	508
Reverse ctagaatgcagcaccaacaaa	Zein	
Forward caacaaagggtaatatcgggaaacctc	CaMV35S	370
Reverse gaagtgacagatagctgggc	CaMV35S	
Forward gccggtcttgcgatgattatc	NOS	190
Reverse catagatgacaccgcgc	NOS	
Forward tcttgtgctgatgaaggtatgtcc	MON810	223
Reverse tcggcagaggcatcttcaa	MON810	
Forward cggccagcaagccttgta	NK603	113
Reverse tcccgactctctcttctcaagca	NK603	

Table II. Variable conditions of PCR applied to target the different sequences (All amplifications were extended at 72°C during 10 min)

	PCR program	Target and cycles	Reference
Activation	95° C, 3 min	5S ribosomal DNA	This manuscript
Amplification	95 °C, 30 sec		
	60 °C, 30 sec	40 cycles	
	72 °C, 1 min		
Activation	95 °C, 3 min	Zein	Hsin-Ying, H.,
Amplification	95 °C, 30 sec		& Tzu-Ming, P.
	60 °C, 1 min	40 cycles	
	72 °C, 1 min		
Activation	95 °C, 3 min	CaMV35S	This manuscript
Amplification	95 °C, 30 sec		

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	50 °C, 1 min	40 cycles	
	72 °C, 1 min		
Activation	95 °C, 30 sec		
	50 °C, 1 min	40 cycles	
	72 °C, 1 min		
Activation	95 °C, 3 min	MON810	Hsin-Ying, H.,
Amplification	95 °C, 1 min		& Tzu-Ming, P
	60 °C, 1 min	40 cycles	
	72°C, 1 min		
Activation	95 °C, 3 min	NK603	Hsin-Ying, H.,
Amplification	95 °C, 1 min		& Tzu-Ming, P
	60 °C, 1 min	40 cycles	
	72 °C, 1 min		

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