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Development of a qPCR method for detection of maize species

Sithuli Dinethya Weerasinghe[1], K.G. Y. O Vidhushani[2] and A.M.M.H Athapaththu[2*]

[1]School of Science, Business Management School (BMS), Sri Lanka

[2]Industrial Technology Institute (ITI), 363 Bauddhaloka Mawatha, Colombo 00700, Sri Lanka

*mashih7@gmail.com

Abstract

Maize is a widely used cereal around the world, and it is essential to know and study more about this species. Therefore, a qPCR technique was carried out to see whether the maize species were present in the sample. The gene that was used as a reference for the maize species was the SSIIb gene. First, the sample of maize was weighed accurately using an analytical balance, and the DNA was extracted by following the QIAGEN-DNeasy mericon food kit procedure. Once the DNA was extracted, it was quantified using the bio-spectrometer to determine the purity and concentration of the extracted DNA. For the amplification of this extracted DNA, specific primers, and probes specific to the SSIIb gene were reconstituted according to the volumes given in the manual, and the PCR master mix was prepared, and qPCR was performed for the 50 ng and 100 ng concentrations and PCR water. The curves obtained for the 50 ng and 100 ng concentrations were smooth curves with similar CT values, showing that the SSIIb gene was present and successfully detected. Overall, the results concluded that the SSIIb gene in maize species was successfully detected through the qPCR method.

Keywords: Maize species, SSIIb gene, qPCR, CT value.

1. Introduction

1.1 Genetically modified crops. Genetically modified (GM) is the gene transfer of organisms using laboratory techniques. These include cloning genes and inserting genes into cells to possess valuable traits such as insect resistance, herbicide resistance, disease resistance, abiotic stress tolerance, and nutritional improvement.[1] The first GM crops introduced were in the mid-1990s in the US., with GM maize being the most genetically modified crop after soybean. These adoptions have shown many benefits, such as reducing pesticide and insecticide use, increasing crop yields, decreasing the cost of crop production, and lowering CO_2 emissions. Along with all these benefits, there can be negative impacts as well, such as concerns about possible allergenicity and toxicity to human beings, potential environmental risks such as adverse effects on non-target organisms, the evolution of resistance in insects and weeds, gene flow, etc.[2] Figure 1 shows examples of genetically modified crops along with their percentage acreage in the US.

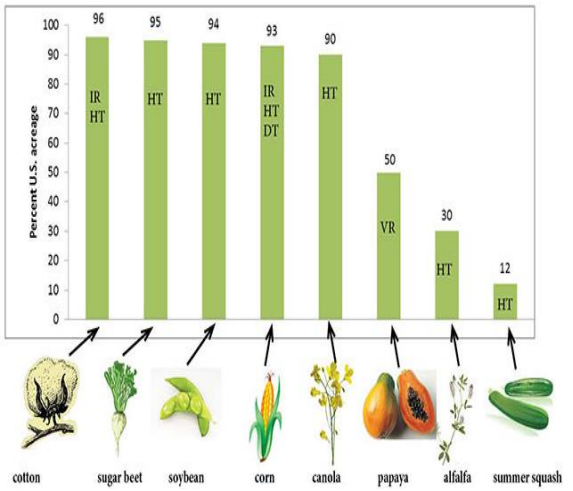

Figure 1. Currently grown GM crops in the US., traits for which they are modified, and percentage of the total acreage of the crop planted to GM varieties.[3]

IR=Insect-resistant, HT=herbicide-tolerant, DT=drought-tolerant: and VR=virus-resistant.

1.2 Maize species. Maize, *Zea mays,* a member of the family true grasses, or *Poaceae*, is a vital cereal grain used worldwide.[4] Maize is one of the most widely produced genetically modified crops. Except in Antarctica, maize can be found on every other continent. There are about 50 species of maize existing in different colours, shapes, sizes, and textures. Most cultivated maize types are yellow, white, and red.[5]

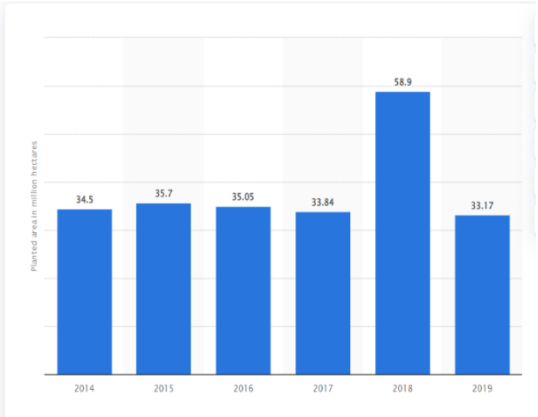

Figure 2. U.S. acreage of genetically modified corn 2013-2019.[6]

Figure 2 represents, the maize planted area in million hectares from 2014 to 2019. The benefits of GM maize include increased grain yields, decreased insect damage and high quality. In short, growing GM maize reduces health risks to consumers and increases the income of farmers.[7] Genetically modified crops are also likely to be crucial in feeding the world's growing food population and improving food security. Along with their many benefits, GM crops can also have disadvantages. Therefore, GM rules and regulations have been established.

1.3 GMO rules and regulations. GMO regulations have been established worldwide in countries, based on economic, societal, and political reasons. A score is set in the GMO index for different countries showing the country-by-country restrictiveness of GMO regulation. The score is based on six main parameters of GMO regulations: risk assessment, labelling, approval process, traceability, coexistence, and membership in international agreements.[8] The US., which is considered the global leader in the commercialization and development of GM crops, holds the global market share in agricultural biotechnology at close to 30%. The US. has no specific, overarching federal law targeted at the regulation of GMOs, unlike most countries. Therefore, GM products are assessed under safety, health, and environmental laws.[9] In Sri Lanka, according to Food Act No. 26 of 1980, no person shall store, import, transport, sell, distribute, or offer for sale any GMOs or any food produced from GMOs without any approval.[10]

1.4 qPCR technique. Many techniques, such as ELISA, multiplex PCR, etc., detect GM crops. However, qPCR testing further increases sensitivity, enabling more efficient testing for large-grain samples and processed foods.[11] The main advantages of qPCR include fast and high-throughput detection, quantification, and a lower time for amplification and visualization.[12]

There are two main methods for qPCR detection, which are the SYBR green assay (non-specific dsDNA binding) and the TaqMan assay. A DNA sequence-specific hydrolysis probe labelled with a fluorophore and a quencher is used in the TaqMan method, as shown below in figure 3. Both assays can be adapted to be absolutely or relatively quantitative, although the TaqMan method is more specific than SYBR green.[13] In this study, we use the TaqMan assay method for the detection of maize species.

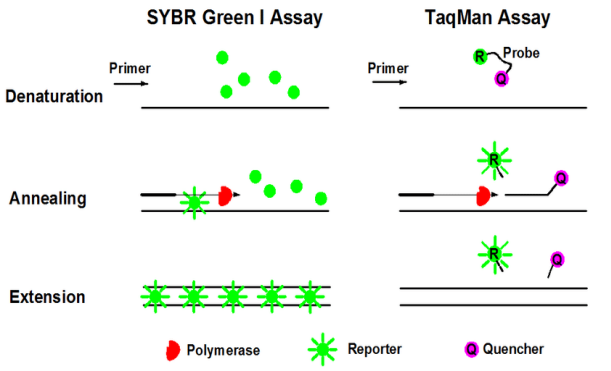

Figure 3. Method of qPCR.[14]

1.5 Significance of the Project. Maize is one of the most widely grown GM crops worldwide, with many benefits. To detect maize, qPCRis widely used, as it is a highly sensitive and quantitative technique for detecting GM crops.

The main objective of this research is to develop a qPCR method for the detection of maize species using maize specific primers. This method can be used to detect taxon specific gene in maize, which can be used to detect maize reference gene in GM maize samples. Therefore, qPCR provides a significant advantage in detecting these GM maize species.

2. Methodology

2.1 DNA extraction using the Dneasy Qiagen Mericon food kit. The workbenches were wiped with 10% bleach followed by 70% ethanol before the procedure. A 0.2006 g of maize sample provided by the biotechnology section was taken for extraction. The DNA extraction was performed according to the manufacturer's guidelines, with modifications.[15]

2.2 Quantification of DNA. The extracted maize DNA was quantified using the Eppendorf bio spectrophotometer. The concentration and purity were analyzed at 260 nm and 280 nm wavelengths. The extracted maize DNA sample was diluted to 50 µg/ml from 87.6 µg/ml. Both the extracted DNA of maize sample and the diluted DNA sample were stored at -20ºC until further use.

2.3 Amplification of maize DNA. PCR was carried out in a volume of 15 µl containing 50 ng and 100ng of extracted sample DNA PCR master mix buffer, 0.5 µM concentration of each (sense and antisense) primer; 0.2 µM probe; the balance consisted of sterile ultrapure water. PCR assays were carried out in Quant studio 5 qPCR system using a standard PCR protocol. The cycling conditions consisted of 2 minutes of UNG treatment at 50ºC, 10 min of initial denaturation at 95 °C, followed by 45 cycles consisting of initial denaturation of 95ºC for 10 minutes, 15 second denaturation at 95 ℃ and an annealing at 60°C for 1 minute.

3. Results

3.1 Quantification by Bio-Spectrometer. The concentration of extracted DNA was 87.6 µg/µl.

Purification for A260/A280- 1.89

Purification for A250/A230- 1.69

3.2 qPCR Results. As shown in figure 4 and 5, amplification curves were obtained for the SSIIb gene with 50 ng and 100 ng concentrations, which were detected and obtained through the qPCR

technique. No amplification curve for the PCR water sample was seen.

As shown in figure 4, the amplification plot was obtained, and the CT value for 100 ng concentration is 25.216.

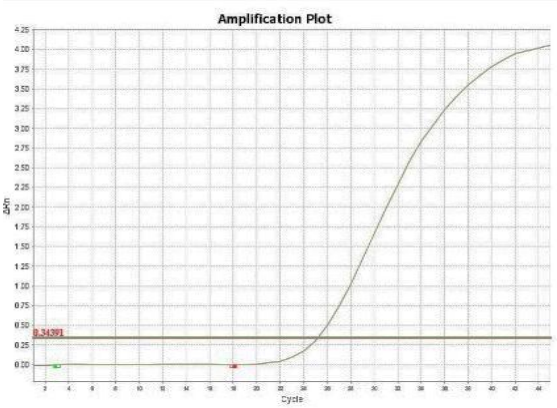

Figure 4. Amplification plot obtained for 100 ng concentration of extracted DNA.

As shown in Figure 5, the CT value obtained for a 50 ng DNA concentration is 25.411.

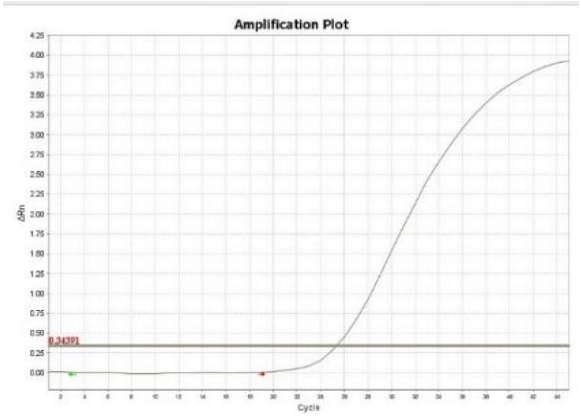

Figure 5. Amplification plot obtained for 50 ng concentration.

As shown in figure 6, no amplification plot was obtained for the sample with PCR water and no extracted DNA.

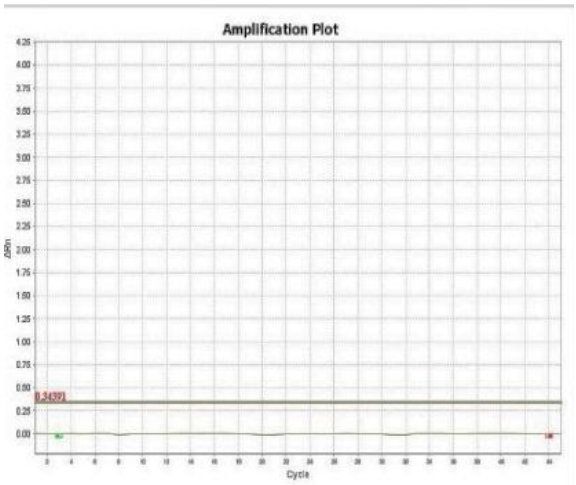

Figure 6. Amplification plot obtained for the PCR water tube.

4. Discussion

Growing maize has been demonstrated to be one of the most economical, feasible, and sustainable approaches for resource-poor farmers and to be environmentally friendly. Therefore, knowledge of the genomic and genetic resources and components of maize species is vital to guide genetic analysis and precision breeding with desirable product profiles for maize varieties.[18]

For dependable and reproducible RT-quantitative PCR (qPCR) analysis, reference genes are frequently utilized to standardize gene expression levels, to perform relative quantification and to use as housekeeping genes.[20] Therefore this study focuses on developing a qPCR-based method to detect SSIIb gene in maize as a reference gene to detect GM maize. The SSIIb gene is a starch synthase gene in maize and is involved in regulating starch content.[24]

Foods are exposed to high pressure, heat, radiation, and pH changes during processing, all of which cause DNA to break down and fragment. The DNeasy mericon Food Kit's refined chemistry was created to recover short

DNA fragments, guaranteeing that even severely fragmented DNA is effectively extracted and amplified in PCR procedures. With these properties, extraction with the DNeasy mericon Food Kit is the first generally applicable extraction technology that provides optimal and accurate results even when utilizing extremely inhibited, highly processed, fatty, acidic, high, or low DNA content foods.[25]

In DNA quantification, the concentration of DNA in the sample was 87.6 µg/µl, showing that DNA was successfully extracted.[16] The purity of the sample was 1.89 for the ratio of A260/A280 absorbance, which is between the ratio for pure nucleic acids of 1.8 and 2.0.[23] This confirms that the extracted DNA was pure and not contaminated.[17]

In DNA quantification, the concentration of DNA in the sample was 87.6 µg/µl, showing that DNA was successfully extracted. The purity of the sample was 1.89 for the ratio of A260/A280 absorbance, which is between the ratio for pure nucleic acids of 1.8-2.0.[23]

The qPCR method developed to detect the maize sample was successful and showed that the SSiib gene was present, as observed by the results. The qPCR results obtained showed that the SSIIb gene was detected for 50 ng and 100 ng sample concentrations, and no amplification curve was seen for the PCR water sample, proving no cross-contaminations or false positives have occurred.[21] However, the CT values obtained for both 50 ng and 100 ng are very similar, with the 50 ng concentration sample being slightly higher comparatively, whereas theoretically, the 50 ng DNA sample should obtain one CT value higher than the 100 ng concentration.[22] This slight variation maybe due to pipetting errors.

Therefore, with the results obtained, the objective of detecting the presence of maize species in the GM maize was successfully achieved by extracting DNA, quantifying the extracted DNA, and amplifying the extracted DNA, by the qPCR method.

This work was only carried out to develop the test method, and for future work, the number of samples used can be further increased, as in our study only 50 ng and 100 ng samples were used to confirm the presence of the SSIIb gene. A dilution series could also be used to achieve a more accurate reading and test for the presence of the SSIIb gene. Furthermore, different varieties of maize species can be used to compare and analyse the genes present in each sample.

5. Conclusion

In conclusion, the results obtained from the qPCR show that the DNA was successfully extracted and quantified. Although the CT values for both 50 ng and 100 ng concentrations obtained were similar, possibly due to a dilution error. We can successfully say that by detecting the SSIIB gene, the presence of the maize species was confirmed using the qPCR technique.

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