ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

DEVELOPMENT OF PLASMID DNA MOLECULES FOR IDENTIFICATION, DETECTION, AND INTEGRATION OF GENES

Raghu bheemanathi, Dr. Syed shahab Ahmad

Research Scholor, Department of biotechnology, Sri sathyasai university of technology and medical sciences, Sehore Bhopal. raghubheemanathi16@gmail.com

Professor, Department of biotechnology, Sri sathyasai university of technology and medical sciences, Sehore Bhopal. info@sssutms.co.in

ABSTRACT

In this research, DNA degradation and PCR amplification of transgenic and nontransgenic maize DNA were investigated under thermal treatment at 100°C and 121° C for 300 min. The influence of different factors has been monitored, namely, temperature, duration of treatment, DNA extraction methods, size and location of amplicons, DNA markers, PCR primers, and GM events. A new PCR-based method targeted at the transgenic Cry1Ab gene has been worked out for the detection of insect resistant GM crops. The obtained results clearly indicate that heating at 100° C has a slight impact on the DNA integrity and PCR amplification. However, 121°C has induced time-dependent degradation of the genomic DNA, and it has considerable influence on the PCR amplification of certain amplicons and may affect GMO detection.the outcomes of this study clearly demonstrate that various processing parameters, such as temperature, duration of exposure, and DNA extraction method, as well as transgenic event, DNA markers, and size and location of the amplicons, are critical factors influencing PCR-based identification of food ingredients and the reliability of GMO detection. The PCR methods applied in the given research show high specificity and sensitivity for the reliable detection of maize and GMOs in processed products.

1. INTRODUCTION

1.1 Introduction

Genetic plasmid expression vectors have gained popularity in the field of gene therapy and immunisation because of their stability, biochemical simplicity, and relative safety when compared to viral vectors. [1,2] Even when administered intravenously, plasmids are poorly absorbed by cells. [3] By using electroporation, plasmid DNA vectors can be improved by several orders of magnitude in terms of their ability to encode transgenes of interest. [4,5,6,7]

When using plasmids for gene therapy, integration of vector DNA into host genomes is a serious concern, even though this is necessary to ensure long-term gene expression, especially in the case of proliferating cell populations. After developing a quantitative gel-purification technique for integration in vivo,[8,9] we used numerous rounds of gel electrophoresis to remove the extrachromosomal plasmid vector from HMW genomic DNA, and then performed a vector-specific PCR to determine whether the vector

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022**

had been successfully integrated. Genome-inactivating mutations are much more likely to arise spontaneously than plasmid DNA integration following intramuscular delivery, as evidenced by the fact that integration was three orders of magnitude less likely (using a worst case scenario). [8,9,10,11] Similar outcomes have been reported by others. [12] As DNA transport efficiency improves and quality is affected by new technologies like electroporation, the frequency of integration might rise. For example, higher integration could help gene therapy targeting a rapidly reproducing cell population that sheds episomal plasmids, but it could also impair the patient population in other ways (eg, for prophylactic vaccines).

Using the mouse EPO gene from the (pCMV/mEPO) plasmid, a prior work by Rizzuto et al.[13] showed that electroporation boosted EPO transgene expression by at least 100-fold and reduced the amount of plasmid needed to induce an increase in hemocrit. There is evidence that electroporation has a direct influence on plasmid entry into the host genome and on plasmid integration into host DNA. A gel purification test was utilised to determine the frequency of integration. Because results from gel-purification assays could be attributed to poor removal of free DNA from HMW genomic DNA, verification of integration would necessitate verification of covalent coupling of plasmid-to-genetic DNA sequences. RAIC, which was designed for this reason, is capable of recognising uncommon single-copy integration events in complicated mixes utilising repetitive-anchored integration capture.

1.2. Plasmids - ancient means of bacterial adaptation and diversification

The ability of bacteria to adapt and evolve is widely credited to horizontal gene transfer via plasmids. When it comes to environmental conditions, bacterial community composition is the most important factor, probably save for broad-host range plasmids that can be found in any environments. Only a small percentage of the population is thought to have plasmids, which would allow it to quickly adapt to changing environmental conditions. Bacterial adaptability in response to numerous selective forces is unquestionably demonstrated by the transmission of antibiotic resistance genes between bacteria of different taxa via plasmids [15,16]. For decades, the molecular biology of some plasmids has been studied, but little attention has been paid to their distribution in the environment, their ecology, and the causes driving their spread and diversification.. Human or plant infections account for an overwhelming majority of plasmid-carrying strain research. Because of the absence of techniques for detecting and quantifying plasmids and for successfully cultivating their hosts, little research has been done on plasmid ecology in natural settings.

More plasmids have been fully characterised in recent decades because of advances in sequencing technology. Studies on plasmid evolution and modularity as well as the presence of hot spots for the insertion of support genes have been discovered by comparative sequence analysis of the plasmid genomes [17,18]. For the creation of primers and probes for plasmid detection or classification, the increasing plasmid sequence database is essential. DNA-based technologies have made it possible to examine and quantify the quantity of plasmids in various environmental samples, without the need for cultivation. An in-depth study of plasmid ecology is essential for a complete picture of how these tiny genetic elements influence the evolution of bacterial life. In particular, it is necessary to investigate variables that enhance plasmid strain proliferation, horizontal gene exchange, as well as the costs and benefits of plasmid carrying to their hosts further. Detection, isolation, and characterization of plasmids and their ecology are the focus of this

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

chapter, which provides a review of current approaches. Recent research will be used to demonstrate the strengths and weaknesses of the approaches used to investigate plasmids, along with some insights gained. Gram-negative and Gram-positive bacteria can use the techniques outlined in this chapter, however the chapter's primary focus is on plasmids in Gram-negative bacteria.

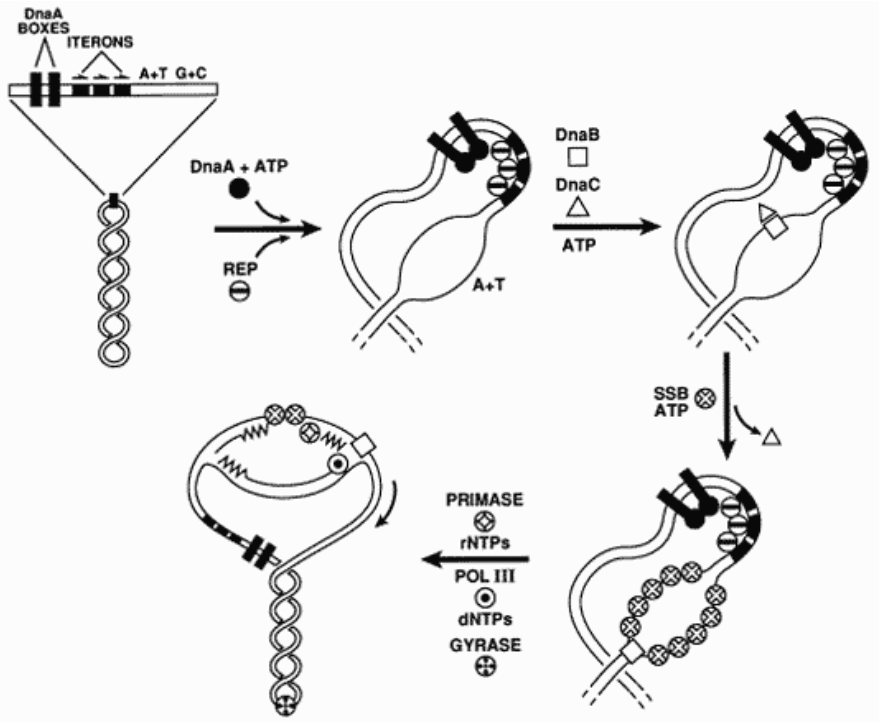

Fig.1. Modelforinitiationofreplicationinaniteron-containingplasmid(Helinskietal,1996).

One of the strands (the leading strand) is continuously synthesising, whereas the other (the lagging strand) is not (Kelman and O'Donnell, 1995). However, the synthesis of the two strands appears to be linked. When DNA replication begins, it can go one way around the plasmid, or both directions at once. In the early phases of leading strand synthesis, some plasmids may require host DNA polymerase I, although the majority of plasmids that replicate via theta method use plasmid-encoded Rep initiator protein.

2. A BRIEF REVIEW OF THE WORK

2.1 General study

There has been a surge in the use of naked DNA transfer for gene therapy to deliver therapeutic genes to cure tumours, genetic abnormalities, and acquired diseases at a low cost (Wang H, 2020). DNA molecules outside of the chromosome, known as plasmids, may self-replicate and can be found in many kinds of bacteria. It is also possible for some plasmids to travel horizontally between neighbouring bacterium cells, where they might transfer genes that aid bacteria in adapting to new settings and severe conditions, therefore propagating vertically throughout the population (Norman et al., 2009).

In the field of horizontal plasmid transfer, conjugation is definitely the most researched technique of plasmid transfer from a donor cell to a recipient cell via conjugative pilus (Smillie et al., 2010). These genes could be used by bacteria to clean heavy metal-polluted environments, or they could be used by bacteria and plants to foster mutualistic relationships (Hall et al., 2015; Wang et al., 2018). As a public health issue, plasmids have the potential to encode hazardous bacteria's virulence or antibiotic resistance determinants

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022**

(Partridge et al., 2018; Pilla and Tang, 2018). Bacterial plasmids are the primary drivers of antibiotic resistance gene transmission, and they have played a major role in the global antimicrobial resistance epidemic (San Millan, 2018).

A plasmid's life is governed by three basic processes: (1) reaching a critical copy number in the host cell by endlessly replicating, (2) partitioning between daughter cells upon division, and, in some cases, (3) transmitting by means of cell-cell contact (see Figure 2.1A). A time-dependent variable, plasmids in each cell can be considered as a result of the molecular interaction between bacterial hosts, plasmids, and the extracellular environment, which causes the plasmid count to fluctuate. Researchers have hypothesised that plasmid replication, partition and transmission comprise a complicated system that can be studied theoretically using methods from dynamical systems and numerical computation.

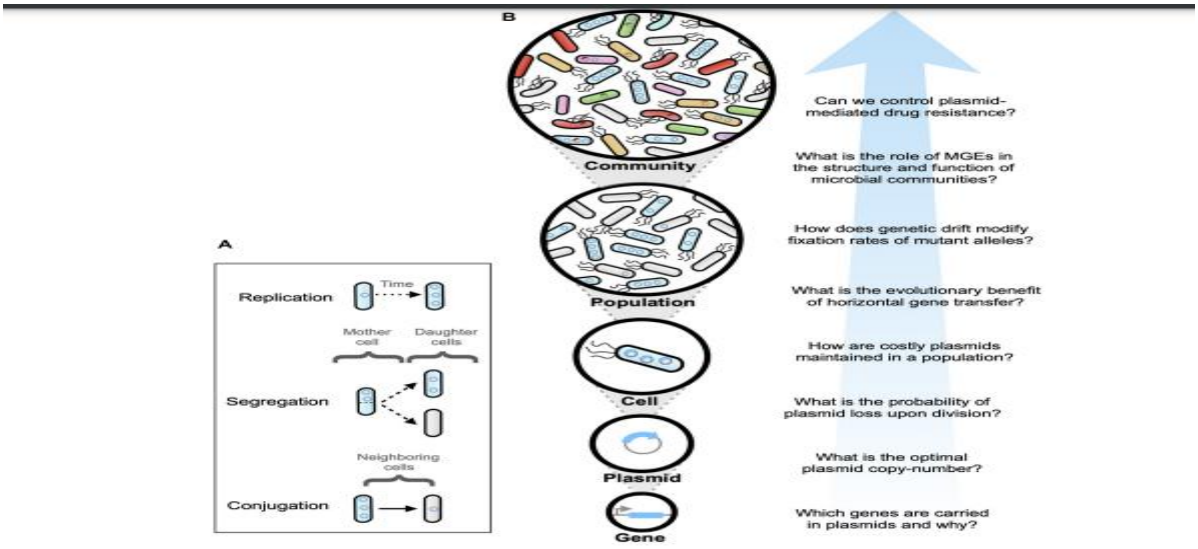

FIGURE 2.1 | (A) As plasmid replication and copy-number control take place inside individual cells, plasmid segregation occurs, and plasmid conjugation occurs, plasmid dynamics emerge (between neighbouring cells). (B) The plasmid life cycle has various levels of complexity, as shown in this schematic diagram. In each level of complexity, mathematical modelling and computer simulations have been used to answer critical concerns.

Models from the 1980s (Nordström et al., 1980; Bremer and Lin-Chao, 1986; Ati and Shulert, 1987; Keasling and Palsson, 1989), for example, calculated the probability of plasmid loss depending on replication method and mean copy number. Mathematical modelling has allowed researchers to evaluate the efficacy and robustness of various plasmid copy-number control systems in the face of environmental perturbations and heterogeneous populations. It was also explored by Nordström et al., Müller and Goss and Peccoud in the 1980s and 1990s for the dynamics of plasmid replication. The role of noise in plasmid replication kinetics was further studied in the 1990s and 2000s by Nordström et al., Müller et al. and Goss and Peccoud (Summers, 1991; Summers et al., 1993). Overall, these studies helped us better understand how different mechanisms of molecular copy-number control and partitioning affect the chance of segregational loss during division.

3. LITERATURE SURVEY

3.1 Plasmid Copy-Number as an Evolvable Trait

It is feasible to reduce the probability of plasmid loss in populations by compensating for plasmid costs or minimisingsegregational

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

loss (e.g., through plasmid addiction systems or active partition mechanisms). The alternative is to increase the amount of plasmid copies in each cell, which can be done even if your plasmid does not include these molecular mechanisms. Another benefit of raising the copy-number may be enhanced stability (e.g., increase in gene dosage, or producing genetic redundancy that enables different versions of plasmid-encoded genes to transiently co-exist in the same cell).

In contrast, the enormous quantities of plasmids might have a detrimental effect on the growth rate of their bacterial hosts. With strong selection against plasmid copy-number increases, it has been shown that this fitness cost vs. plasmid stability tradeoff leads to an optimal plasmid copy-number that can be modified by evolution in vitro and theoretically (Mei et al., 2019).

It has been hypothesised that ecological cycles between plasmids with low, moderate, and high copy numbers can possibly increase plasmid stability through selection against plasmids with extreme copy numbers (Watve et al., 2010).

One of the most critical aspects of plasmid evolution is the link between the amount of plasmid copies and their ability to evolve. There is a direct correlation between the number of copies of plasmid-encoded genes and their mutational target size, which means that the greater the number of copies of plasmids, the greater the possibility that a mutant form will arise (San Millan et al., 2016; Dimitriu et al., 2020).

For the study of plasmid evolution, Ilhan and his colleagues (2019) used a combination of experimental evolution and the typical haploid form of the Wright-Fisher model, which includes plasmids (Peng and Kimmel, 2005). In a forward-time simulation of the model with constant population size, genetic drift, selection coefficient, and starting mutant frequency were utilised to estimate allele frequencies through time. In addition, by testing the stability and evolvability of plasmids with various PCNs, the researchers showed that mutations in plasmid-encoded genes are sensitive to segregational drift purification from the population. For large-copy-number plasmids, the time to fixation grows as the number of mutations decreases, which was confirmed by both experimental evolution and the sequencing of the genome.

3.2 Fitness Cost Compensation

Furthermore, theoretical studies have demonstrated that alternative evolutionary mechanisms promote long-term plasmid stability in addition to tweaking PCN to balance the danger of segregational loss and the expense associated with growing plasmid concentration. Theoretically and empirically, it has been demonstrated that plasmid stability can be improved by compensatory evolution that lowers the cost of plasmid carriage and, as a result, lessens selection against plasmids.

In order to model the evolutionary dynamics of a megaplasmid with a mercury-resistant cassette and the transposition of resistance genes into the chromosome, researchers Harrison et al. (2016) employed a computational model. Computed models reveal that plasmid cost compensation increases stability of plasmids, as well as aiding in the fixation of accessory features on bacteria's genomes. Using this model as a starting point, the researchers found that the most likely long-term solution to stable bacteria-plasmid partnerships is to reduce the costs of plasmid transmission (Hall et al., 2017).

4. PROPOSED METHODOLOGY

4.1 Materials and methods

Qiagen supplied the Taq PCR core kit and RNase A. (GmbH, Hilden, Germany). Invitrogen Corporation provided the pRSET C vector (Carlsbad, CA, USA). Agarose came from Amresco in Solon, Ohio, the USA. GenEluteTM Plant Genomic DNA Miniprep extraction kit was supplied by Bangalore, India-based Sigma Aldrich. MaximaTM SYBR Green/ROX qPCR mastermix kit, mass

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

loading dye (6), and a 100 bp DNA ladder were provided by Glen Burnie, MD, USA The 1 kb DNA ladder was provided by Fermentas, the United States, and Bangalore Genei, India. Kits from Qiagen and Applied Biosystems were used for extraction and purification, and the TaqMan PCR Core Kit was purchased from Qiagen GmbH, Hilden, Germany. TaqMan PCR Core Kit In this experiment, pre-stained markers and restriction enzymes from New England Biolabs (Beverly, MA, USA) were used. The ISO 21569 method was followed. Reverse transcription and amplification of DNA was performed using a pre-heated CTAB extraction buffer (CTAB-20 mg/L, NaCl, Tris-0.1 moles, Na DNA ligase from USB Corporation in Cleveland, OH, USA). They purchased Hiveg peptone and fresh deodorising pearls from Himedia laboratories PVT. Ltd. Mumbai in India, and sterilised disposable petri plates from Himedia laboratories PVT. Ltd. It was necessary to buy a FastPlasmidTM Mini Kit from Eppendorf India Limited, based in Chennai, India. Invitrogen Life Technologies in Grant Island, New York, provided the plasmid vector pRSET C and the E. coli strain DH5.

4.2 Isolation of Genomic DNA

Methods for purifying DNA rely on the molecule's unique chemical properties, namely its length and negative charge, to set it apart from other cellular molecules. In order to isolate pure DNA from a tissue sample, cells are ground or lysed in an aqueous solution that protects the DNA while destroying other cell components (Figure 4.1). The detergents in this list denature proteins and dissolve lipid membranes. To stabilise and separate negatively charged DNA from proteins such as histones, cations such as Na+ are used. Nuclease inhibitors require Mg2+ ions as a co-factor, so chelating agents such as EDTA are added to protect DNA (enzymes that digest DNA). Free, double-stranded DNA molecules can be released from chromatin by using an extraction buffer that contains proteins and other cellular components.

There are a variety of ways to isolate the free DNA molecules. Precipitation of proteins is the most common method for removing them from a sample. It is mixed with ethanol to precipitate the DNA. EDTA and a pH buffer can be added to water to dissolve the DNA, which can then be used in other reactions after the ethanol has been removed from the DNA sample. There are additional techniques, like PCR, that can be used to isolate specific genes or DNA fragments from tissue samples.

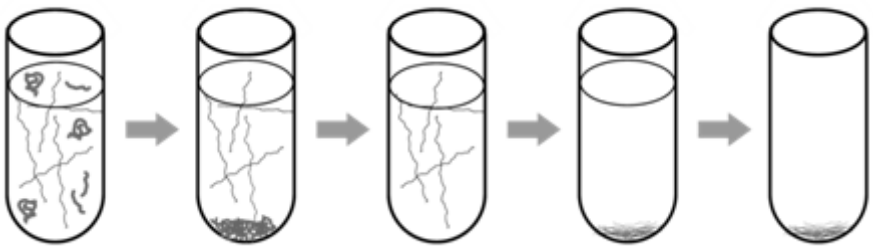

Figure 4.1: Precise DNA extraction by successive precipitation out of solubilized cell constituents. Proteins are precipitated, followed by the precipitation of DNA (from the supernatant) in ethanol, resulting in a DNA pellet. (Original-Deyholos-CC:AN)

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Method 1:

ISO 21569's CTAB method was followed. Carbamazepine, NaCl, Tris, and DNA Ligase were purchased from USB Corporation in Cleveland, Ohio. Samples were suspended in 1.5 mL of preheated CTAB extraction buffer. From Himedia laboratories PVT. Ltd. Mumbai, India: Agar type-I, tryptone type-I, culture tubes, fresh deodorising pearls, Hiveg peptone, sterile disposable petri plates Eppendorf India Limited, Chennai, India, supplied the FastPlasmidTM Mini Kit. This experiment was performed using Invitrogen Life Technologies' pRSET C vector and the E. coli strain DH5 from Grant Island, New York, the United States. It's time to talk about methodology. 2 EDTA-0.02 mol/L pH 8.0) and incubated at 65°C for 30 minutes to obtain genomic DNA. After 30 minutes of incubation at 65°C with 15 L of amylase and 10 L of RNase A (10mg/mL), 15 L of Proteinase K (20mg/mL) was added and incubated for another 45 minutes at 65°C. For ten minutes, the samples were spun at 12000 rpm in a centrifuge. The supernatant was dissolved in chloroform and centrifuged at 12,000 rpm for 15 minutes before the chloroform was added. Incubation was carried out for one hour at room temperature, followed by a 15-minute centrifugation at 12,000 g and collection of the supernatant, which was diluted twofold with precipitation buffer (CTAB-5g/L, NaCl-0.4 mol/L). 1.2 M NaCl and 350 L chloroform were combined, centrifuged at 12,000 rpm for 10 minutes, and the results were collected. It took 20 minutes at 25+2°C and 15 minutes at 12,000g to separate the isopropanol from the aqueous layer after which the sample was centrifuged for 15 minutes. Ethanol was used to wash the sample in order to remove DNA from the supernatant. A sterile milli Q water solution was used to store the DNA after drying it for 5 minutes at 65°C.

Method 2:

In accordance with the manufacturer's instructions, the Gene Elute Plant genomic DNA miniprep kit (Sigma Aldrich, USA) was used to extract and purify plant genomic DNA. Powder sample was mixed with lysis solution A and solution B in a 2 mL micro centrifuge tube, and centrifuged at 14,000 revolutions per minute. This mixture was incubated on ice for 5 minutes with RNase A (10mg/mL) and precipitation solution (370 L). After centrifuging at 12000g for five minutes, the supernatant was filtered through a filter column. The DNA binding column was loaded with 500 microliters of column preparation solution. The flow through was discarded after spinning for one minute. It was necessary to add 700 µL of binding solution to the sample filtrate before it could be transferred to the DNA binding column and thoroughly mixed by inversion. Using wash solution, the column was thoroughly cleaned twice before being transferred to a new 1.5 mL tube. Pre-heated to 65 °C, 100 L of elution solution was used to remove the bound DNA.

4.3 Initial development of DNA extraction techniques

Friedrich Miescher discovered DNA in cells while working on a project related to cell chemistry. The leukocytes he collected from fresh surgical bandages were used in 1869 to purify and classify proteins. "Nuclein" was the name of a new substance discovered during his experiments in the nuclei. On the basis of his discoveries, he devised two protocols for isolating the novel compound that has come to be recognised today as DNA from proteins and other cellular constituents. Felix Hoppe-Seyler and his mentor In 1871, Felix Hoppe-Seyler published this scientific finding and the isolation protocols used.. In spite of this, Meselson and Stahl's DNA extraction method was not widely used until 1958. Centrifugation was used to extract DNA from E. coli bacteria samples using salt density gradients. A variety of biological sources have been able to be

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

extracted using the same techniques since then.

In order to extract DNA from a sample, various extraction methods follow a set of common procedures, including cell disruption, nucleoprotein complex denaturation, nuclease inactivation, removal of contaminant DNA, and finally precipitation. Centrifugation and a variety of organic and nonorganic reagents are commonly used in the basic steps of most of these assays. Automated procedures and commercially available kits have resulted as a result.

5. EXPECTED OUT COME OF THE PROPOSED WORK

Insect-resistant Bt corn varieties, Bt-176 and MON810, were analysed for transgenic events. Additionally, MON810 is the only transgenic cultivar that is grown in European countries. GM maize and unmodified maize DNA were compared in each experiment. Thermal treatment, genomic DNA extraction, DNA assessment by agarose gel electrophoresis and spectrophotometer, and PCR amplification of GMO-specific amplicons were all sequential steps in the analytical procedure (7). GMO detection has been studied as a result of the combination of several important factors. Nontransgenic and transgenic maize events; endogenous and exogenous DNA markers; PCR primers; amplicons' size and location were all factors in this study. Combinations of the following were used: The CTAB method was used to extract wild type and event Bt176 maize from 100°C to 121°C, while the DN easy plant kit was used to extract wild type and event MON810 maize from 121°C to 100°C. There have been no previous studies of these GM crops and various factors under the processing conditions mentioned.

Table 5.1: Oligonucleotide primers.

Target	Primers	Sequence	Ampacon(bp)
Maizezeingene	ZEINI/ZEINr	ACACCACCGACCATGGCAGC	102
Maizeinvertasegene	IVRI/IVRr	TCTCCCGTGATCCTGCCCCG	140
Maizeinvertasegene	IVTASI/IVTAS2	CCGCTGTATCACAAGGGCTGGTACC	226
CrylAbgene	Crylf/Crylr	GCACCTCCGTGGTGAAGGGC	258
CaMV35Spromoter	P3581/P35Sr	CGTGCACCATGATGTGTGATTCGAC	
CrylAbgene	Cry1241/Cryl24f	CATCCTCAACAGCATCACTATCT	124

5.1 Impact of Heat on Genomic DNA.

As commonly used temperatures in food production and preservation, we used 100°C and 121°C for thermal treatment. Pasteurization is done at 100°C, whereas sterilisation is done at 121°C for some products. Thermal treatment has a significant effect on genomic DNA if the temperature and duration of exposure are high enough (Figure 5.1).

Processing samples at different temperatures yielded distinct results. 100°C had a much smaller effect on DNA integrity than 121°C. All untreated samples had the high-intensity genomic DNA bands with a high molecular weight. Figure 5.1(a) shows that the flour treated at 100°C contained large amounts of the entire genomic DNA, with only minor smearing. When processing samples at 121°C, DNA extracts from these samples contained

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

strong smeared DNA bands, but the size of the fragments was reduced (Figures 5.1(b)–5.1(d)). As a result, the findings show that high temperatures have an adverse effect on DNA integrity. Furthermore, 121°C exposure degraded the material more severely than 100°C heating. Samples obtained using the DNeasy kit had a greater impact than those obtained using the CTAB method (Figures 5.1(a) and 5.1(b)), likely because of the kits' different approach to DNA purification (Figures 5.1(c) and 5.1(d)). Non-transgenic and transgenic varieties were found to have no significant differences.

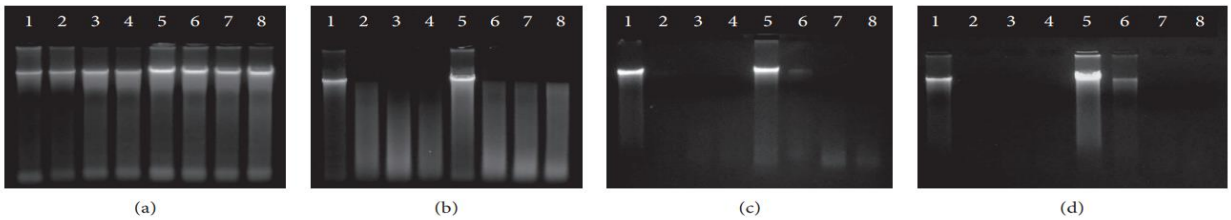

Figure 5.1: Genomic DNAs from the samples treated at high temperature: (a) 100°C and (b–d) 121°C. Exposure duration: lanes 1 and 5 =0 min; lanes 2 and 6= 60 min; lanes 3 and 7 =180 min; lanes 4 and 8 = 300 min. Extraction was performed by the CTAB method (a, b) and DNeasy kit (c, d). Samples: wild-type maize (lanes 1–4 in (a–d)), event Bt176 (lanes 5–8 in (a–c)), and event MON810 (lanes 5–8 in (d)).

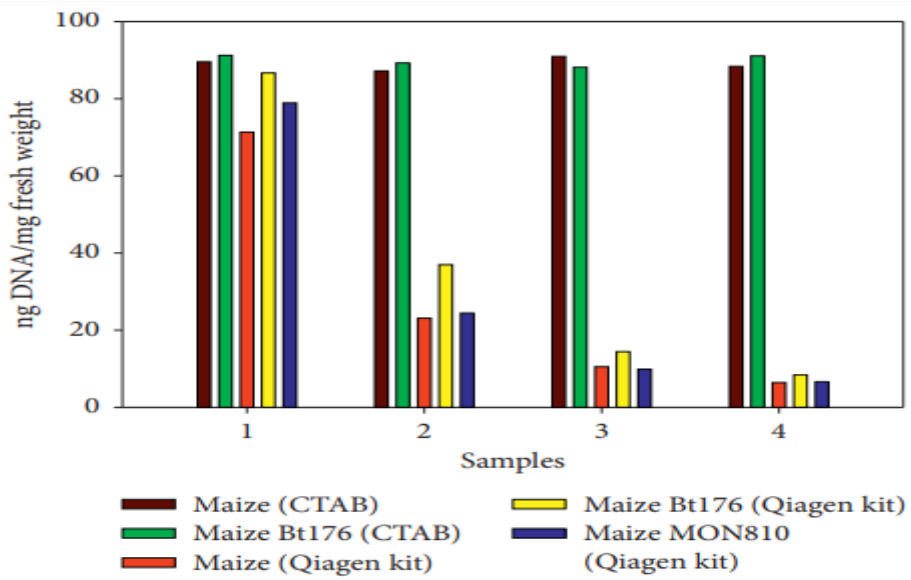

Figure 5.2: Spectrophotometric analysis of DNA extracted from samples: wild-type maize by the CTAB method = maize (CTAB) and DNeasy kit = maize (Qiagen kit), event Bt176 by the CTAB method = maize Bt176 (CTAB) and DNeasy kit = maize Bt176 (Qiagen kit), and event MON810 by the DNeasy kit = maize MON810 (Qiagen kit) treated at 121°C. Exposure duration: 1 = 0 min; 2 = 60 min; 3 =180 min; 4 = 300 min.

Analyses using spectrophotometry showed that the extraction method used to obtain the DNA had a significant impact on genomic DNA yield (Figure 5.2). DNA was more easily extracted using the CTAB method than the DNeasy kit. The highly processed samples showed a particularly notable difference. A 300-minute thermal treatment at 121°C had no

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

effect on the CTAB method's DNA extraction yield for either wild type or GM maize (event Bt176). However, the DNeasy kit's DNA extraction yield was drastically reduced when heated to 121°C (Figure 5.2). In addition, the DNA yield decreased dramatically with increasing exposure time for both wild type and GM events (Bt176 and MON810). When compared to untreated samples, DNA yield decreased by 50–60% after 60 minutes and by 90% after 300 minutes. This could be explained by the fact that CTAB extracts contain all of the DNA fragments generated during processing, whereas the DNeasy kit's DNA extracts remove short DNA fragments generated after thermal degradation. Thus, the CTAB method is better suited for DNA extraction from processed foodstuffs than the DNeasy kit. - DNA integrity and quantity are influenced by the temperature and duration of thermal processing, as well as the DNA extraction method. These results are in line with those from the previous research.

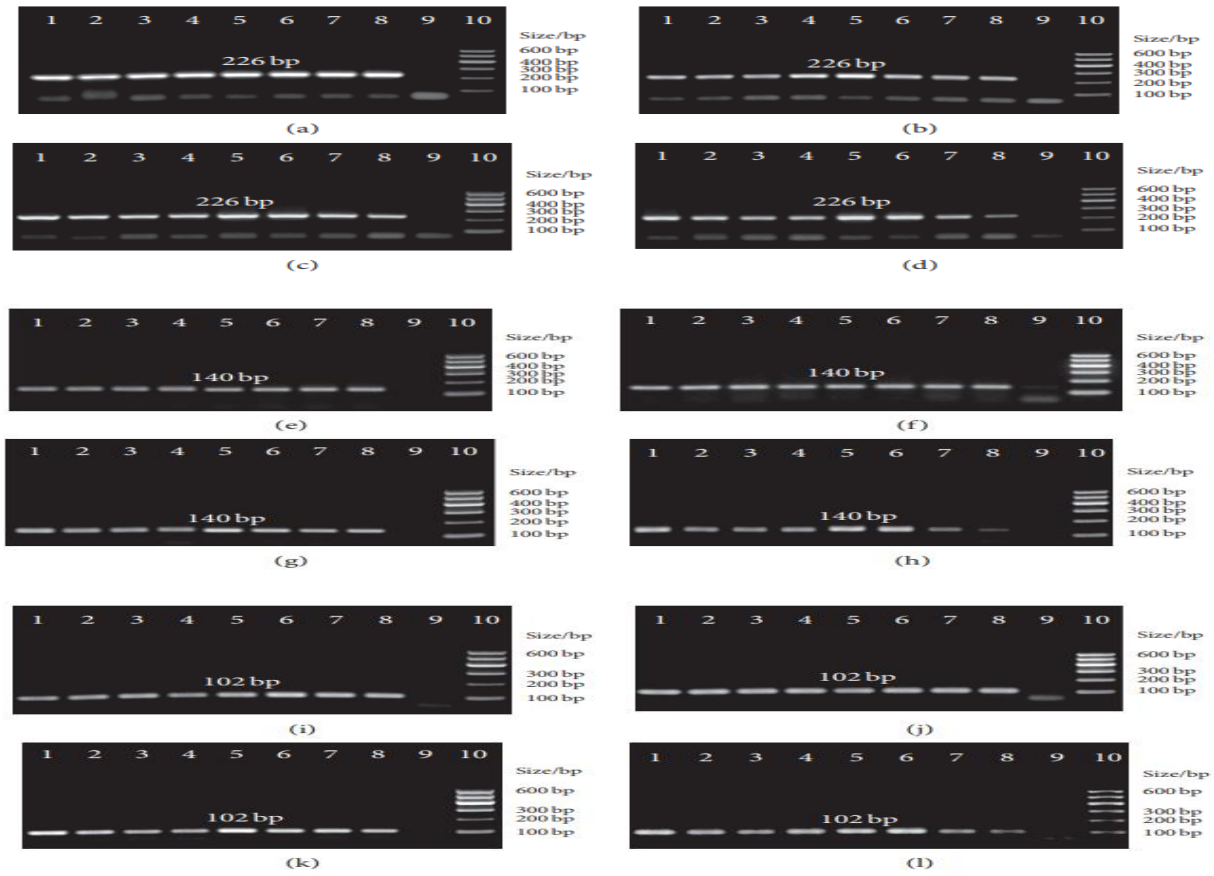

Figure 5.3: PCR analysis of endogenous amplicons with size of (a–d) 226 bp, (e–h) 140 bp, and (i–l) 102 bp from the samples treated at high temperature: 100°C (a, e, i) and 121°C (b–d, f–h, j–l). Exposure duration: lanes 1 and 5 = 0 min; lanes 2 and 6 = 60 min; lanes 3 and 7 =180 min; lanes 4 and 8 = 300 min. Extraction was performed by the CTAB method (a–b, e–f, i–j) and DNeasy kit (c, d, g, h, k, l). Samples: wild-type maize (lanes 1–4 in (a–l)), event Bt176 (lanes 5–8 in (a–c, e–g, i–k)), event MON810 (lanes 5–8 in (d, h, l)), water (lane 9 in (a–l)), and GelPilot 100 bp ladder (Qiagen) (lane 10 in (a–d)).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

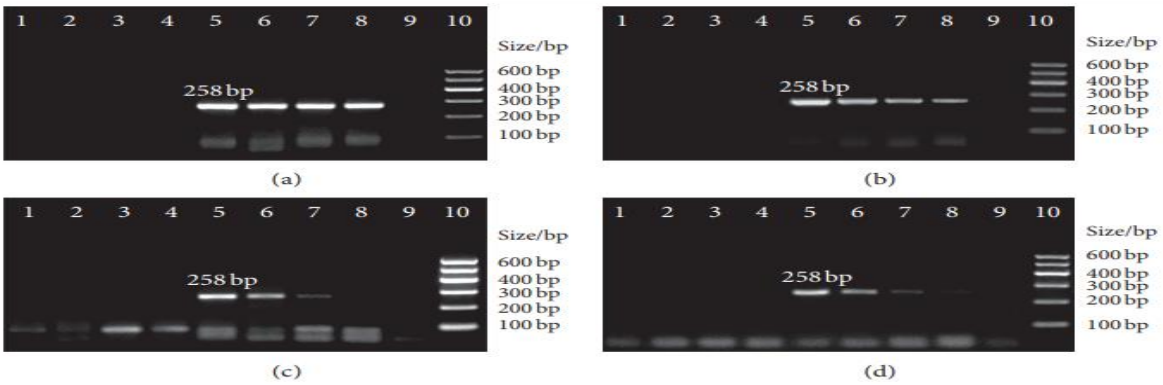

Figure 5.4: PCR analysis of exogenous 258 bp amplicon from the samples treated at high temperature: (a) 100°C and (b–d) 121°C. Exposure duration: lanes 1 and 5 = 0 min; lanes 2 and 6 =60 min; lanes 3 and 7 =180 min; lanes 4 and 8 = 300 min. Extraction was performed by the CTAB method (a, b) and DNeasy kit (c, d). Samples: wild-type maize (lanes 1–4 in (a–d)), event Bt176 (lanes 5–8 in (a–c)), event MON810 (lanes 5–8 in (d)), water (lane 9 in (a–d)), and GelPilot 100 bp ladder (Qiagen) (lane 10 in (a–d)).

CONCLUSION

Overall, the multiple displacement amplification method provided the greatest range of resistance plasmids from the investigated cecal samples. However, due to the inconsistencies of the results and the difficulties experienced with this method, it is not the ideal protocol to use when working with a large volume of samples under short deadlines. The commercial kits, alkaline lysis method and TRACA did not provide a wide range of resistance plasmids from our sample compared to the others tested. Therefore, the exogenous plasmid isolation method resulted in the widest range of resistance plasmids with ease of application and consistency across samples. While this method relies on the conjugative ability of the plasmids present, it is both an efficient (plasmids can be obtained in a short time-frame) and effective (a good range of plasmids can be acquired) method which worked with all of the cecal samples tested. Therefore, we recommend the exogenous plasmid isolation method when extracting antibiotic resistance plasmids of clinical relevance from a large number of complex samples.

REFERENCES

1. Herweijer H, Wolff JA . Progress and prospects: naked DNA gene transfer and therapy. Gene Therapy 2003; 10: 453–458.
2. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA . DNA vaccines. Annu Rev Immunol 1997; 15: 617–648.
3. Nishikawa M, Huang L . Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum Gene Ther 2001; 12: 861–870.
4. Bigey P, Bureau MF, Scherman D . In vivo plasmid DNA electrotransfer. CurrOpinBiotechnol 2002; 13: 443–447.
5. Gehl J . Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand 2003; 177: 437–447.
6. Trezise AEO . In vivo DNA electrotransfer. DNA Cell Biol 2002; 21: 869–877.
7. Fattori E, La-Monica N, Ciliberto G, Toniatti C . Electro-gene-transfer: a new approach for muscle gene delivery. Somat Cell Mol Genet 2002; 27: 75–83.
8. Nichols WW, Ledwith BJ, Manam SV, Troilo PJ . Potential DNA vaccine

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved**, **UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022**

integration into host cell genome. Ann NY Acad Sci 1995; 772: 30–39.

9. Ledwith BJ et al. Plasmid DNA vaccines: assay for integration into host genomic DNA. Dev Biol (Basel) 2000; 104: 33–43.
10. Ledwith BJ et al. Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. Intervirology 2000; 43: 258–272.
11. Manam S et al. Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. Intervirology 2000; 43: 273–281.
12. Martin T et al. Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. Hum Gene Ther 1999; 10: 759–768.
13. Rizzuto G et al. Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation. Proc Natl Acad Sci USA 1999; 96: 6417–6422.
14. Wang H, Zhang Y, Huang B, Deng W, Quan Y, Wang W, et al. Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. Cell [Internet]. 2020 Jun 6 [cited 2020 Jun 8];0(0).
15. Ahmad, A., Græsbøll, K., Christiansen, C. E., Toft, N., Matthews, L., and Nielsen, S. S. (2015). Pharmacokinetic-pharmacodynamic model to evaluate intramuscular tetracycline treatment protocols to prevent antimicrobial resistance in pigs. *Antimicrob. Agents Chemother.* 59, 1634–1642. doi: 10.1128/AAC.03919-14