Molecular Detection of Transgenes in improved varieties of Cowpea

ABSTRACT

Cowpea is one of the most consumed food in Africa. Recombinant DNA technology has been used to insert foreign genes into plants genome via cassettes there by creating a new generation of plants with desired traits. Such plants possess improved seed quality, yield and resistance to pests and pathogens. The aim of this study is to detect transgene in Cowpea using molecular analysis. Improved varieties of Cowpea were obtained from IITA while local varieties were purchased from Terminus Market in Jos. All samples were screened for the presence of transgene via DNA extraction and PCR. Results shows the presence of transgene in MKT1, IT89KD and IT99KD, CAMV, NOS and VIP3BA respectively and none was found in local varieties. Improved varieties were found to have higher protein content than local variety.

Keywords: Recombinant DNA, Transgenes, Cassettes

INTRODUCTION

Recombinant DNA technology has brought about several useful products, not limited to genetically modified plants like cotton, corn, soya beans, etc. but also other vital biomedical products like the human growth hormone, insulin, etc. (James, 2014). Plants are designed to possess improved seed quality, yield and resistance to pests as well as pathogens (Boldura and Popescu, 2016).

The resulting modified end-product(s) have been documented to contain foreign genetic material like a desired gene, introduced or inserted via specific transgenic cassettes into the hosts genome, thereby creating a new generation of plants for example with a desired trait, thereby making the new product phenotypically different to its native counterpart (Fraiture *et al.,* 2015).

Whilst there are a lot of scientific validation for the direct application of genetic modification (GM) technology towards food security to combat hunger and starvation, coupled with the propagation of pathogen, drought and heat resistant crops as part of the accolades for this technique, new health issues pertaining to both human and environmental health have raised concerns about the futility of GM products (Sateesh, 2008). With increased public health

awareness on the use of genetically modified organisms, concerns have been raised pertaining to environmental and food safety. The recent upsurge in different cancer types, introduction of known and new trans-species allergens as well as toxins, emergence of antibiotic resistance pathogens via unintended transgenesis via pollination, etc. represent just some of the issues pertaining to the use and consumption of GM foods in society. This has therefore necessitated the assessment in one part of GM products qualitatively (Amiri *et al.,* 2013).

Polymerase chain reaction (PCR) technology serves as a gold standard for the primary screening of the presence of foreign genes in products or food crops in the absence of GM labelling. This is due to the flexibility, specificity and sensitivity of the technique (Li *et al.,* 2012). To this end, GM assessment consist of the screening for regulatory elements associated with genetically modified organisms (GMO) such as specific promoter and terminator sequences (Forte *et al.,* 2005).

Nigeria is the largest producer and consumer of cowpea grain, with about 5 million hectares and over 2million metric tonnes production annually, followed by Niger (650,000 mt) and Brazil (490,000 mt) (Singh.2002). A long-term drought in the Sahelian zone of West Africa has caused many farmers in this part of Africa to shift more of their production to cowpea because of its drought tolerance (Duivenbooden *et al.* 2002).

Considering the status of the global food chain, and the importation of a substantial proportion of Nigeria's food supply, it is very likely that GMOs are present in a significant portion of foods sold in Nigeria. Currently, a number of countries are actively pursuing detection and monitoring of GM products in food commodities, including Malaysia, Brazil, China, and Saudi Arabia (Abdullah *et al.,* 2005). At the same time, a number of countries in the world have put in place laws or regulations requiring transparency regarding the presence of GMOs or derivatives of GMOs in food products (Okpara *et al.,* 2016). To date, the actual status of GMO in foods sold in the Nigerian market cannot be ascertain, because no testing has been carried out to empirically assess foods in the marketplace.

Aim

The aim of this study is to detect transgene in Cowpea using molecular analysis

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Jos, Plateau State. It is located in Central Nigeria and lies within Latitude of 9 55' N and Longitude 8 53' 31.63''E, distinguished by its high bounding scarp and by bare grassland, covering about 3,000 sq miles. It is bounded to the North East by Bauchi State, bounded to the North West by Kaduna State, bounded to the South West by Nasarawa State and South East by Taraba State, with an estimated Population of 1,000,000 inhabitants.

3.2 Sample Collection

Local Cowpea was purchased from terminus market in Jos metropolis, Jos North LGA of Plateau State and prepared for analysis. Improved varieties of Cowpea, MKT1, IT99K and IT89K were obtained from the National Biosafety Management Agency Abuja through the Institute for Agricultural Research Zaria (IAR). Both samples were transported to the Biochemistry Division of National Veterinary Research Institute Jos for analysis.

3.3 Screening for GM using PCR

3.3.1 DNA Extraction

Genomic DNA extraction was carried out using the commercially available Zymo Research Plant and Seed Extraction Kits following the manufacturer's recommended procedure. Alternatively, a modified CTAB method (Osuji *et al.,* 2016) was employed for genomic DNA extraction. Approximately 700 µl CTAB buffer was employed for the initial incubation, 500 µl isopropanol for DNA precipitation and 100 µl 70% ethanol for the two washing steps. Finally, extracted DNA sample was dissolved in 200 µl TE buffer supplemented with 10 mg/ml RNase (2 µl). Purity and quantification of DNA sample was assessed using a UV spectrophotometer. The DNA sample would be stored at -20°C until used for PCR.

3.3.2 PCR Analysis

Four Primers were used in this research, which include, ***VM*** for detection of species-specific DNA, p***35s*** Specific for the 35S promoter of the Cauliflower Mosaic Virus, ***TNOs*** Specific of the terminator of the Nopaline synthase gene from *Agrobacterium tumefaciens*. ***Vip3*** Specific for the ***Vip3A and B*** transgene coding for a *Bacillus thuringiensis* insecticidal gene.
Primers were adopted from Datukishvili *et al.* (2015). Primers were purchased in lyophilized form and reconstituted as directed by the manufacturer.

The PCR reaction mix (20 µl) constitute of 12 µl OneTaq Quick Load PCR Master Mix (New England Biolabs; 20 mM TrisHCl, 1.8 mM MgCl2, 200 µM dNTPs), 1 µlTaq DNA

polymerase (25 units/ml), 1 µl bovine serum albumin (10 mg/ml), 5 µl template DNA and 1 µl each of forward and reverse primers.

Reactions was carried out in triplicates in order to confirm amplifications. PCR reactions was performed using a thermo-cycler. The PCR cycling condition involves an initial denaturation of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, extension at 72°C for 35 s and a final extension step at 72°C for 5 min (Osuji *et al.*, 2016). The PCR products was resolved on 1.5% agarose gel by using ethidium bromide or SYBr green/safe (Osuji *et al.*, 2016). The electrophoresed gels were examined under ultra violet trans illuminator and photographed using a Gel Documentation System (GDS).

Table 1 : List of primers along with sequences

Primers	**GENE**	**Sequences**	**Expected amplicon (Bandsize)**	**Reference**
CaMV P35S	Cauli Flower Mosaic virus promoter sequence	F-CGTGCACCATGATGTGTGATTCGAC R- GTGGGATTGTGCGTCATCCCTT	195 bp	Datukishvili *et al.* (2015).
NOS terminator	Nopaline Synthase terminator sequence	F-GGTACCGGATCCAATTCCCGATCGTT R-CGCGCTATATTTTGTTTTCTATCGCGT	118 bp	Datukishvili *et al.* (2015).
Vip3Ab	Vegetative Insecticidal protein gene	ATTTTGCTACGTCTAATTGCTCGTCGT ACGT	1350 bp	Li *et al.* (2012).
VM	Vigna Microsatelite	F:CATTGCCACCTGGTTTCAC R:GAGGCTCAGCATTTGTTT	220 bp	Ali *et al*, 2015

RESULTS

1.1 Results of Screening and Detection of GM cowpea

The extracted DNA was of good quality with purity in the range of 1.70 to 2.11. The genetic material suffered minimal breakage during extraction with a good yield as sufficient quantities

was obtained for downstream applications.Amplicon of lecithin gene was detected at band size approximately 330 bp**.** The results showed that all the varieties of both local and improved Cowpea contain lecithin which is specific for cowpea.

Results of the screening shows that transgenes were detected in all the three (3) improved cowpea varieties. The genes include the CaMV 335 S promoter, NOS terminator and vip3AB. However, no transgene was detected in the local varieties of cowpea. Plates I to V show the gel electrophoresis analysis of the cowpea varieties.

Plate I: Genomic DNA bands of cowpea varieties on 1%agarose gel at 90 volts for 20 min, DNA samples were extracted by the ZR Plant/Seed MiniPrepKit.

KEY: S: 10,000bp DNA Ladder, Lane 1: *Vignaunguiculata* (IT99K-573-1);

Lane 2: *Vignaunguiculata* (MKT); Lane 3: *Vignaunguiculata* (UAM09-1051-1);

Lane 4: *Vignaunguiculata* (IT89KD-288); Lane 5: *Vignaunguiculata* (Milk);

and Lane 6. *Vignaunguiculata* (Canonola).

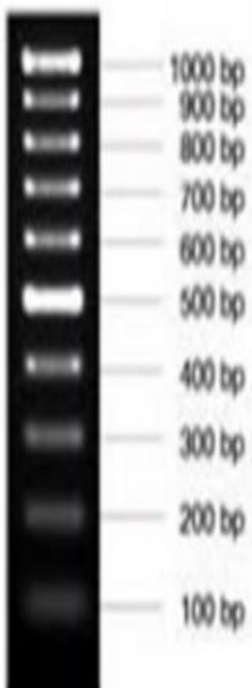

Plate II: Amplicon of *V. unguiculata lecithin gene* with VM primers approximately 330 bp

KEY: S: 1,000bp DNA Ladder; lane 1: Molecular ladder;

Lane 2: *Vignaunguiculata* (IT99k-573-1); lane 3: *Vignaunguiculata* (IT89KD-288); lane 4: *Vignaunguiculata* (UAM09-1051-1); lane 5: *Vignaunguiculata* (MKT); lane 6: *Vignaunguiculata* (Milk); lane 7: *Vignaunguiculata* (Canonola).

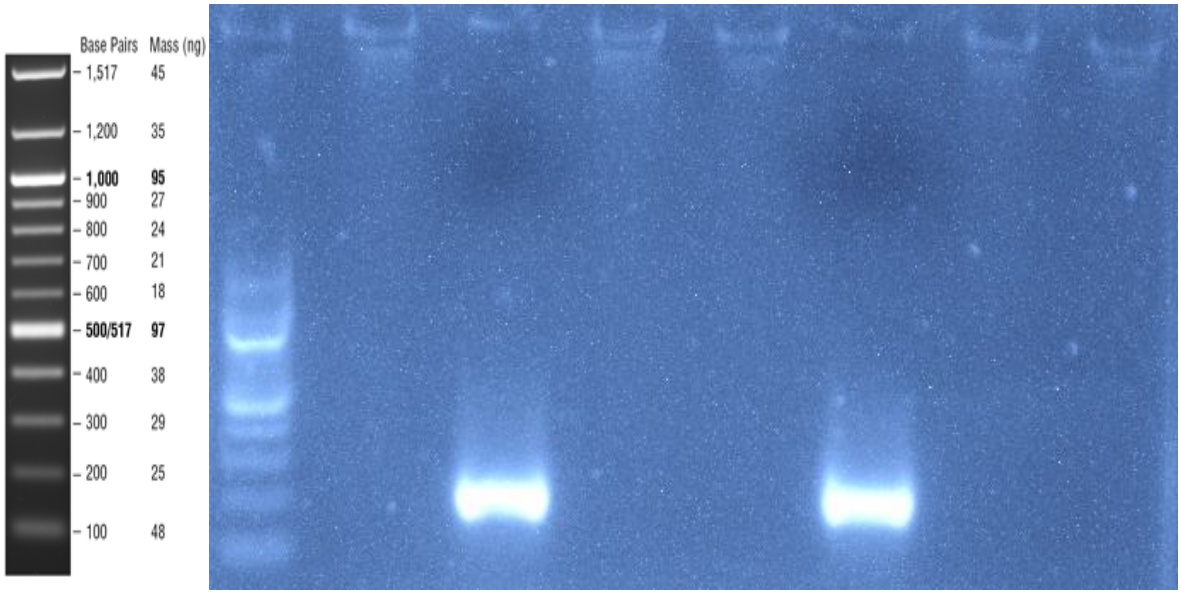

Plate III: Amplified *V. unguiculata NOS* terminator region approximately 195 bp.

KEY: lane 1: S (Molecular ladder); lane 2: *Vigna unguiculata* (IT99k-573-1); lane 3: *Vigna unguiculata* (IT89KD-288) lane 4: *Vigna unguiculata* (UAM09-1051-1); lane 5: *Vigna unguiculata* (MKT); lane 6: *Vigna unguiculata* (Milk); and lane 7: *Vigna unguiculata* (Canonola).

Plate IV: Amplified *V. unguiculata CAMV 35 S* promoter region approximately 118 bp

KEY: lane 1: S (Moleculaar ladder); lane 2: *Vignaunguiculata*(IT99k-573-1);
lane 3: *Vignaunguiculata*(IT89KD-288); lane 4: *Vignaunguiculata*(UAM09-1051-1);
lane 5: *Vignaunguiculata*(MKT); lane 6: *Vignaunguiculata*(Milk);
lane 7: V*ignaunguiculata*(Canonola).

Plate V: Amplicon *V. unguiculata* Vip3BA gene approximately 400 bp

KEY: lane 1: S (Molecular Ladder); lane 2: *Vignaunguiculata*(IT99k-573-1);
lane 3: *Vignaunguiculata*(IT89KD-288); lane 4: *Vignaunguiculata*(UAM09-1051-1);
lane 5: *Vignaunguiculata*(MKT); lane 6: *Vignaunguiculata*(Milk); lane 7: *Vignaunguiculata*(Canonola).

Discussion

In this study, species specific genes were detected in both improved and local variety of of cowpea. These findings are in agreement with the work of Barbau-Piednoir *et al.* (2009), Xu *et al.* (2014) and Jasur *et al.* (2020) amongst several investigational screening studies. The

absorbance ratio to quality control and concentration of extracted DNA for sample types ranges between 1.7 and 1.9 with this amount is acceptable for PCR amplification based on previous studies (Gryson *et al.,* 2007; Guertler *et al.,* 2013; Safaei *et al.,* 2019). The genomic DNA extraction results are in conformation with procedural steps of screening for GMOs in food samples (Tripathi, 2005; Querci and Mazzara, 2011; Kamle *et al.,* 2017).

All the 6 samples which tested positive for species specific DNA using VM primer indicates the presence of GMO targets.This screening for GMO is based on the specific detection of most common regulatory sequences, the CaMV 35S promoter and *nos* terminator (Querci and Mazzara, 2011), as the selected genes are not crop specific and can detect the GM elements even in raw and processed matrices like food and feed products developed from GM crops (Shahid *et al.,* 2019; Deisingh and Badrie, 2005).

The CaMV 35S promoter sequences was observed only in two DNA improved variety samples (IT89K and MKT) while only IT89K showed positive for tNOS. The CaMV 35S promoter analysis showed that 33.33% of the samples are GM positive which agrees with the work of Arun *et al.,* (2013) where 25% of the 100 samples detected by PCR technique are GM positive and Oraby *et al.* (2005) who reported that 12.5% of the food product tested gave the positive result to CaMV 35S, while negative results were determined for NOS primer. Similarly, Erkan and Dastan (2017) reported that 11 samples of the rice and rice flour products analyzed contained GM targets. Safaei *et al.* (2019) reported 2 out of 81 (2.4%), testing positive for CaMV35S while no positive result was detected for the NOS terminator. Saadedin *et al.* (2019) in a study to screen for GM tomatoes reported 15 out of 78 (19.23%) samples were positive for CaMV-35s promoter while 16.67% (13 out of 78) samples positive for tNOS.

Vip3 gene (3A and 3B) was selected for the identification step in the general screening of GM foods. IT99K and MKT which is 33.33% (2 out of 6) samples were positive for the presence of Vip3 genes. The results agree with the report by Sahin *et al.* (2018) who detected the vip3 in 18 out of 80 Bt crops and further showed the protein expressed to be effective against insects. Also, this result is similar with the reports from previous studies screening for *vip3* genes showing a variable range of incidence, with percentages such as 49% by Bergamasco *et al.* (2013), 69.3% by Baranek *et al.* (2015) and 87% by Djenane *et al.* (2017). The *vip3* genes were targeted as these are known to have insecticidal activity against certain lepidopteran pests (Estruch *et al.* 1996; Bett *et al.,* 2017). Furthermore, transgenic cotton expressing a *vip3A* gene

was protected against the cotton boll weevil (CBW) (Lepidoptera) (Wu *et al.* 2011). The vip3 gene shown to be dominated by *vip3A* genes and *vip3B* genes were detected in this study,this agrees with the work of Bett *et al.* (2017) in accordance with the Bt database (Crickmore *et al.* 2014) where the majority of *vip3* genes belong to the A family, with fewer members in the B family and very few in family C.

The detection of the transgenic cowpea implies the presence of GM in Nigerian markets and consumed as part of staple food. Genetically modified cowpea was accepted in Nigeria amidst concerns due to the consequences of genetically modified crops on health and the environment, which include pesticide resistance, implication of the crops for farmers, and the crops role in feeding the world (Newswire, 2013) despite the country's dire need for biotechnology application to boost food production and quality.

The results of the screening of commercially available cowpea in local markets from Jos showed the widespread availability of GM cowpea and foods in Nigeria and provide insight as to the advantages of GM usage in the agricultural sector by improving and increasing productivity of staple foods and further impacting on the socioeconomic status of Nigerian farmers combating the effects of pests which could lead to loss of yield up to 80% if no control measures are employed, especially the larva is the most destructive stage of this pest and feeds on flower parts, green pods and seeds of cowpea and several other leguminous crops (Singh and Van Emden 1979; Jackai and Daoust 1986;Bett *et al.*, 2017). GM crops (foods) are claimed to provide humans and animals alike superior nutritional value such as higher protein and vitamin content and relatively free from macro-mineral deficiencies affecting wild varieties (Lu, 2005).

CONCLUSION

In conclusion, the improved variety of cowpea confirmed presence CAMV35, NOS, Vip3BA in MKT1, IT89K and IT99K but absent in local cowpea.

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