



## Qualitative Screening of Cry1Ac and Cry2Ab Genes in Nigerian Cotton Genotypes Prior to Commercial Release

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### Abstract

A study was conducted during the 2016 dry season at the Institute for Agricultural Research (IAR), Samaru, in the Northern Guinea Savannah of Nigeria to screen fifty-one (51) cotton (*Gossypium hirsutum* L.) genotypes for the presence of Cry1Ac and Cry2Ab genes. These Bt-derived genes encode insecticidal proteins that provide resistance against bollworms, major pests of cotton. Two qualitative detection methods were employed: Bt lateral flow strips for Cry1Ac and ELISA for Cry2Ab. The genetic materials tested included multi-adversity lines, northern zone lines, eastern zone lines, commercial varieties, and Sabuwa lines. Of the 51 genotypes screened, only two Sabuwa lines tested positive for both Cry1Ac and Cry2Ab, while all others were negative. Limited to qualitative detection, the study nonetheless provides the first baseline evidence of Bt gene presence in Nigerian cotton germplasm prior to the 2018 official approval of Bt cotton. These findings underscore important biosafety and regulatory concerns, highlighting the need for systematic monitoring, enhanced seed certification, and regional harmonization to safeguard varietal integrity and compliance with international biosafety standards.

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### 1.0 Introduction

Cotton (*Gossypium spp.*) is one of the world's most important fibre crops, cultivated widely for its industrial and commercial uses (Jans *et al.*,

2021). Global production is concentrated in tropical and subtropical regions, with China, India, the United States, Brazil, and Pakistan

among the leading producers (Voora *et al.*, 2020). In Nigeria, cotton remains a strategic crop for the agricultural and textile sectors, though production has declined due to pest pressure, poor input systems, and reduced cultivation area (Adeoti *et al.*, 2020; Akanbi *et al.*, 2018).

Lepidopteran pests such as the cotton bollworm (*Helicoverpa armigera*), pink bollworm (*Pectinophora gossypiella*), and spotted bollworm (*Earias* spp.) are major constraints to cotton productivity worldwide (Liao *et al.*, 2022; Manickam *et al.*, 2013). To mitigate these challenges, genetically modified (GM) cotton varieties expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) have been adopted globally. Two Cry genes, Cry1Ac and Cry2Ab,

play a central role (see Table 1): Cry1Ac provides broad lepidopteran control, while Cry2Ab complements its efficacy and helps delay resistance development (Muzaffar *et al.*, 2015; Rajamohan *et al.*, 1998).

Nigeria officially approved Bt cotton varieties (MRC 7377 BG II and MRC 7361 BG II) in 2018 (NBMA, 2018). However, regional adoption patterns and informal seed movement raised concerns that Bt traits might have entered Nigerian germplasm earlier through porous borders and unregulated seed trade (Adenle, 2014; Ramaswami *et al.*, 2012). At that time, no published evidence confirmed such introgression.

**Table 1:** Cry genes and their insecticidal activity (adapted from TNAU Agritech portal, 2023)

Gene	Crystal shape	Protein size (KD)	Insect activity
Cry I A(a), A(b), A(c), B, C, D, E, F, G	Bipyramidal	130 – 138	Lepidopteran larvae
Cry II (Sub-group) A, B, C	Cuboidal	69 – 71	Lepidopterans, dipterans
Cry III (Sub-group) A, B, C	Flat irregular	73 – 74	Coleopterans
Cry IV (Sub-group) A, B, C, D	Bipyramidal	73 – 134	Dipterans
Cry V – IX	Various	35 – 129	Various

Unregulated dissemination of transgenes poses biosafety challenges, including risks to seed system integrity, biodiversity, and international trade compliance (Adenle, 2014, Quist & Chapela, 2001). Robust detection methods such as lateral flow strips and ELISA are therefore essential tools for biosafety monitoring (Celia & Khetarpal, 2006; Lequin, 2005).

This study provides the first baseline evidence of Cry1Ac and Cry2Ab genes in Nigerian cotton germplasm prior to official release. By documenting Bt gene presence in entries collected in 2016, it fills a critical gap in biosafety knowledge and highlights the need for routine seed testing, certification, and regional harmonization of GMO monitoring.

## 2.0 Materials and methods

### 2.1 Study site

The study was conducted during the 2016 dry season at the Institute for Agricultural Research (IAR), Samaru (11°11'N, 7°38'E), located in the Northern Guinea Savannah zone of Nigeria. Field trials were complemented with screen house irrigation to ensure uniform plant growth and availability of tissues for laboratory assays.

### 2.2 Plant materials

A total of fifty-one (51) cotton (*Gossypium hirsutum* L.) entries were evaluated, comprising multi-adversity lines, northern zone lines, eastern zone lines, commercial varieties, and Sabuwa lines (see Table 2). Seeds were obtained from the Cotton Improvement Program of the

Institute for Agricultural Research (IAR), Samaru, and collaborating sources.

### 2.3 Detection assays

Qualitative detection of Bt genes was performed using commercial kits (Envirologix, Portland, ME, USA; supplied by CICR, Nagpur, India), following manufacturer protocols.

**Cry1Ac (Bt-strip assay):** At four weeks after planting, six leaf discs (1 cm diameter) were collected from three plants per genotype. Extracts were prepared in the supplied buffer, and strips were immersed to allow capillary

flow. A single band (control line only) indicated absence of Cry1Ac, while two bands (control + test line) indicated presence.

**Cry2Ab (ELISA assay):** At maturity, six seeds per genotype were halved to expose embryos. Seed tissue was placed in 96-well plates and treated with 50 µL substrate and 50 µL developer solution per well. Plates were incubated in the dark until reaction stabilized. A distinct blue colour signified Cry2Ab presence, while absence of colour indicated a negative result.

**Table 2:** Background of cotton genetic materials studied

S/N	Genotypes	Origin	No. of Entries
1	Multi-adversity lines	IAR Samaru	20 entries
2	Northern zone lines	IAR Samaru	10 entries
3	Eastern zone lines	IAR Samaru	10 entries
4	Commercial varieties	IAR Samaru	6 entries
5	Sabuwa lines	Various parts of Nigeria	5 entries

### 2.4 Replication and validation

Each genotype was analysed using three independent biological replicates, and each replicate was tested in technical duplicate. For Cry1Ac, each replicate consisted of two leaf discs, while for Cry2Ab, two halved seeds were used. Ambiguous outcomes were re-tested until consistent results were obtained. Positive and negative controls provided with the kits were included in every assay to ensure reliability.

### 2.5 Data analysis

Results were recorded qualitatively as positive (+) or negative (–) in accordance with the manufacturer's interpretation guidelines. No spectrophotometric quantification of ELISA reactions was performed. This limitation is acknowledged in the Discussion, and future studies are recommended to incorporate quantitative approaches.

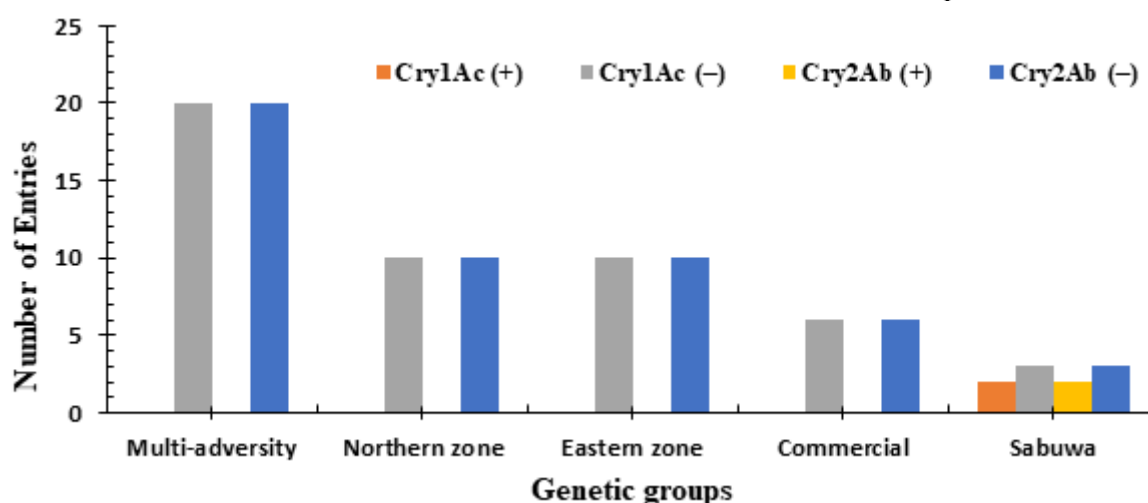
### 3.0 Results and Discussion

The fifty-one cotton genotypes evaluated across five genetic groups (multi-adversity lines, northern zone lines, eastern zone lines, commercial varieties, and Sabuwa lines) were screened for the presence of Cry1Ac and Cry2Ab using Bt-strips and ELISA kits. Results showed that all entries from the multi-adversity (20), northern zone (10), eastern zone (10), and commercial (6) groups were negative, while two of the five Sabuwa lines tested positive as summarised in Table 3.

Negative samples consistently displayed a single band at the top of the Bt-strip (indicating absence of Cry1Ac) and no colour reaction in ELISA (indicating absence of Cry2Ab). In contrast, the two Sabuwa lines tested positive in both assays, with Bt-strips showing two bands and ELISA producing a blue colour reaction, consistent across replicates. These outcomes are summarized in Figure 1.

**Table 3:** Screening outcomes of 51 Nigerian cotton genotypes for Cry1Ac and Cry2Ab genes

Genetic group	No. of entries	Cry1Ac (+)	Cry1Ac (–)	Cry2Ab (+)	Cry2Ab (–)	Notes
Multi-adversity	20	0	20	0	20	All negative
Northern zone	10	0	10	0	10	All negative
Eastern zone	10	0	10	0	10	All negative
Commercial	6	0	6	0	6	All negative
Sabuwa	5	2	3	2	3	Two positives
Total	51	2	49	2	49	Only Sabuwa lines positive

**Figure 1:** Screening outcomes of cotton genotypes for Cry1Ac (Bt-strip) and Cry2Ab (ELISA). Bars show the number of entries in each genetic group classified as positive (+) or negative (–).

The agreement between strip and ELISA results demonstrates the reliability of the detection methods. Similar validation of ELISA against strip-based assays has been reported in transgenic plants (Karcher, 2002), supporting the robustness of this combined approach. Earlier studies have also emphasized the sensitivity and reproducibility of ELISA for detecting Bt proteins (Celia & Khetarpal, 2006; Lequin, 2005). Although representative images of Bt-strip bands and ELISA plates are not included due to data loss, all interpretations were standardized against positive and negative controls, and repeat testing was conducted to ensure reliability.

Globally, Bt cotton has been widely adopted for its effectiveness against lepidopteran pests (Liao

*et al.*, 2022; Manickam *et al.*, 2013). In India and China, large-scale use enhanced yields and reduced pesticide costs (Muzaffar *et al.*, 2015; OFAB, 2012). In West Africa, Burkina Faso's initial adoption was later reconsidered due to concerns over fibre quality and biosafety (Sanou *et al.*, 2019). In Nigeria, the detection of Bt-positive Sabuwa entries collected in 2016—two years before official approval in 2018 (NBMA, 2018)—suggests that transgenic traits had already entered local germplasm, likely through informal seed exchange and porous borders (Adenle, 2014; Daily Trust, 2023; Ramaswami *et al.*, 2012).

The detection of Bt-positive genotypes prior to official release raises critical biosafety and regulatory concerns. The unregulated

dissemination of genetically modified material can compromise seed system integrity, pose ecological risks, and threaten compliance with international trade standards. Similar challenges have been reported in other developing countries with weak regulatory systems that permitted the spread of unauthorized GMOs (Adenle, 2014; Quist & Chapela, 2001; Ramaswami *et al.*, 2012). These findings underscore the need to strengthen Nigeria's biosafety framework, particularly the implementation of the National Biosafety Management Agency (NBMA) Act of 2015. Enhancing border surveillance, enforcing routine seed testing, and promoting regional harmonization will be critical for effective monitoring and traceability.

A limitation of this study is that the assays provided only qualitative presence/absence outcomes, without quantification of Bt protein expression levels. While suitable for baseline screening, this approach does not capture variation in gene expression across genotypes. To mitigate this, multiple biological replicates and technical duplicates were used, and ambiguous samples were re-analysed until consistent results were obtained. Future studies should incorporate quantitative assays such as real-time PCR and quantitative ELISA to complement qualitative screening and provide deeper insights into gene expression and stability.

Although limited to qualitative detection, this study contributes valuable baseline evidence for Nigerian cotton. Broader surveys are warranted to determine the extent of unregulated Bt cotton dissemination nationwide, while investment in local capacity for molecular detection will strengthen Nigeria's ability to monitor and regulate GM crops effectively.

## 4.0 Conclusion

### 4.1 Key findings

This study screened fifty-one cotton (*Gossypium hirsutum* L.) genotypes from Nigeria using Bt-strips and ELISA assays for the presence of

Cry1Ac and Cry2Ab genes. All entries from multi-adversity, northern zone, eastern zone, and commercial groups were negative, while two Sabuwa lines tested positive for both genes. This provides the first baseline evidence of Bt gene presence in Nigerian cotton germplasm prior to the country's official approval of Bt cotton in 2018.

### 4.2 Limitations

The assays employed were qualitative, detecting only the presence or absence of Cry proteins. Quantitative variation in expression across genotypes was not measured. Additionally, representative images of strips and ELISA plates could not be included due to data loss. These limitations reduce the depth of characterization but do not undermine the reliability of the findings, as multiple replicates, technical duplicates, and positive/negative controls were used to validate results.

### 4.3 Recommendations

The detection of Bt-positive genotypes before official release underscores the need for enhanced seed certification systems, border surveillance, and biosafety monitoring in Nigeria. Future studies should expand sampling to include wider germplasm collections and employ quantitative assays such as qPCR and quantitative ELISA to complement rapid screening. Building local capacity for molecular detection and promoting regional harmonization of biosafety frameworks will be critical to safeguard varietal integrity, ecological safety, and compliance with international trade standards.

### Declarations

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**Consent for publication:** Not applicable.

**Availability of data and materials:** Available from the corresponding author upon reasonable request.

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**Authors' contributions:** Ibrahim Abubakar Zango conceptualized the study, conducted the field experiment, and drafted the manuscript. Abdullahi Ibn Yahaya supervised the work and contributed to refining the draft. Mohammed Sagir Mohammed critically reviewed the paper and provided valuable inputs. All authors read and approved the final version.

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