APPLICATION FOR DELIBERATE RELEASE OF GENETICALLY MODIFIED ORGANISM IN ETHIOPIA

APPLICATION FOR DELIBERATE RELEASE OF GENETICALLY MODIFIED MAIZE WITH TRAITS FOR RESISTANCE TO FALL ARMYWORM AND STEM BORER PESTS IN ETHIOPIA

Data protection:

This application contains scientific data and other information which are protected in accordance with the Biosafety Amendment Proclamation No. 896/2015.

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EXECUTIVE SUMMARY

Maize is a critical crop for Ethiopia, ranking as the third most important cereal after tef and wheat. It contributes significantly to the country's food security, accounting for 36% of the total grain production. Annually, Ethiopia produces about 4.2 million tons of maize, cultivated by nearly 9 million smallholder farmers. As the most widely grown cereal in the country, maize plays a vital role in sustaining livelihoods, but its production faces several challenges, particularly from insect pests. Among the most damaging pests are the Fall Armyworm (FAW) and stem borers such as *Busseola fusca* and *Sesamia calamistis*. FAW, a pest originally from the Americas, has become a major problem in sub-Saharan Africa, including Ethiopia. The larvae of FAW consume maize leaves, stems, and ears, severely affecting crop health and causing yield losses that can reach up to 70%. The presence of FAW exacerbates the impact of drought conditions in some regions, compounding the difficulties faced by farmers. To combat these pests, integrated pest management (IPM) strategies are essential. IPM involves a combination of methods, including biological control, the use of resistant maize varieties, and chemical treatments. Early detection and monitoring are crucial to minimize pest damage. One innovative approach gaining attention is the use of Bt (Bacillus thuringiensis) maize, a genetically modified variety that produces insecticidal proteins to protect against pests like stem borers and FAW without harming beneficial insects.

The Ethiopian Institute of Agricultural Research (EIAR) under the TELA Maize project successfully carried out confined field trials with six Bt maize varieties containing event MON89034 to test efficacy against FAW and stem borers in Wondogenet, Melkassa and Bako, between 2023 and 2024, which have shown promise in managing pests like FAW and stem borers. These genetically engineered maize varieties express proteins that target a broad spectrum of pests, providing long-lasting protection and potentially reducing the reliance on chemical pesticides. In addition to broad spectrum on insect control, the combination of Cry1A.105 and Cry2Ab2 insecticidal proteins in a single maize variety, MON89034 provides a much more effective insect resistance. Compared to the maize varieties used as standard check, the Bt maize hybrids have 24.7q/ha average yield advantage. Moreover, the FAW leaf damage score is below 3 as compared to the conventional hybrids showed higher value (>6.0). By improving pest control and enhancing agricultural productivity, these efforts aim to strengthen food security in the country. In the face of climate change and increasing pest pressures, the adoption of pest-resistant maize varieties like Bt maize offers a promising solution to safeguard Ethiopia's maize production and support the livelihoods of millions of smallholder farmers.

The post-release environmental monitoring plan, resistance management plan, and reporting intervals will be made following established scientific procedures and EPAs' guidelines. With this application, approval is sought for the deliberate release of four genetically modified maize varieties with traits for resistance to FAW and stem borer pests in Ethiopia under the Biosafety Amendment Proclamation No. 896/2015.

PART ONE: GENERAL

3.0 General Information

3(1). Name, Address and Contact Details of Applicant	
Ethiopian Institute of Agricultural Research Tel: (+251) 112610090 Fax: (+251) 112370006 P.O. Box: 2003, Addis Ababa, Ethiopia	
Date of Submission:	April, 2025

3(2). Name and field of studies of persons responsible

for planning and carrying out the implementation of the Project, including those responsible for monitoring and safety, in particular the name of the responsible scientist.

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PART TWO: INFORMATION ON DONOR RECIPIENT AND THE MODIFIED ORGANISM

4. Description of the Donor. Recipient and the Modified Organisms

4(1). Scientific name, and when available also common name of the donor organism;

The transgenic maize proposed for deliberate release in this application bears single event MON89034 with traits for insect protection. The trait for insect protection contains *Cry1A.105* and *Cry2Ab2* genes, both obtained from *Bacillus thuringiensis*.

4(2). Scientific name, and when available also common name of the recipient modified organism;

Common	Maize or Corn
Scientific	*Zea mays* L.
Commercial	TELA maize
Transformation event Code	MON 89034
Identification Code registered with the Information Exchange Mechanism	Unique identifier: **MON**-89Ø34-3
Electronic address of the Information Exchange Mechanism	https://bch.cbd.int

4(3). Scientific names, and when available also common names, of species that are related to any of the organisms from which any of the transgenes in the expression cassette have been taken;

The transgenes in the expression cassette are sourced from the soil-borne bacteria called *Bacillus thuringiensis, commonly called (Bt).*

4(4). Degree of relatedness between the donor and recipient (between parental) organisms;

The donor organisms are unicellular prokaryotes and are, therefore, not closely related to the recipient organism, maize that is multicellular with cells that have a nucleus enclosed within membranes and other organelles.

4(5). Description of the geographic distribution and natural habitats of the donor and recipient (between parental) organisms; the environments where they occur.

i) Donor Organisms

Bacillus thuringiensis* (*Bt*) with *Cry1A.105 and Cry2Ab2 genes

Bacillus thuringiensis (Bt) is a spore-forming, Gram-positive bacterium naturally found in soil, with strains used in the U.S. since 1958 for insecticidal products (US EPA, 1988). The geographic distribution of Bt ranges from North America, Central and Sout America, Europe, Africa, Asia, and Oceania indicating its ubiquitous presence in the world (Fig 1). Bt produces protein crystals, or Cry proteins, during sporulation, which are toxic to certain insect pests. These crystals, observed through phase-contrast microscopy, are highly specific and can target various pests in agriculture and forestry. Bt's environmental safety and efficacy make it a valuable alternative to chemical pesticides, offering potential for managing insect resistance to chemicals (Sanchis et al., 2008; Roh et al., 2007). Regulatory agencies like the EPA have determined that Bt products pose no significant risks to human health or the environment (US EPA, 1998).

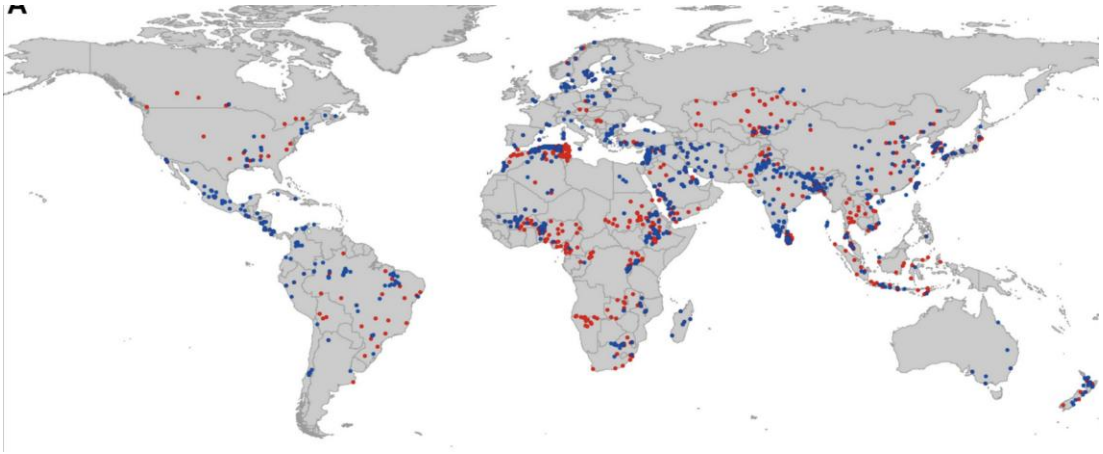


Figure 1. The global distribution of *Bt* (depicted with blue dots). Source: Ejaz at al 2025.

ii) Recipient Organism

Maize (Zea mays) is believed to have been domesticated in Central America around 7,000 to 10,000 years ago (Beadle, 1978) and has since become widely cultivated across diverse climates globally. It originated in the highlands of Mexico, but due to its extensive domestication, maize has lost the ability to survive in the wild and requires human intervention for seed dissemination (OECD, 2003). It is unable to reproduce outside of cultivation and is non-invasive in natural habitats. Maize thrives best in regions between latitudes 30° and 55°, with optimal conditions for growth including a warm monthly temperature range of 21-27°C and a frost-free season lasting 120 to 180 days, with a rainfall threshold of around 150mm (OECD, 2003).

Maize varieties with genetic modifications, such as MON 89034, have been developed in the USA and later approved for cultivation in other countries, including South Africa and Nigeria. In Ethiopia, maize with the MON 810 event, which provides protection against stem borers, has been approved, though it does not address the major threat of Fall Armyworm (FAW), a pest causing significant yield losses in Africa. Efforts to develop maize varieties with better FAW resistance have been ongoing, with Bayer laboratories contributing to the introgression of these traits into maize varieties suitable for local conditions. The Ethiopian Institute of Agricultural Research (EIAR), in collaboration with partners like CIMMYT and Bayer, conducted field trials at Melkassa, Wondogenet and Bako during 2023-2024 to assess the effectiveness of these transgenic maize varieties for insect protection (see section 10(2)).

5. Characteristics of nucleic acids that affect trait expression

5(1). Description of each expression cassette;

The MON 89034 expression cassette refers to the specific genetic sequence within genetically modified maize with event MON 89034 and includes *Cry1A.105* and *Cry2Ab2* genes that encodes, a crystal protein that confers protection against certain lepidopteran pests. The genes are controlled by the 35S promoter which ensures strong expression of the *Cry* genes.

5(2). Whether the nucleic acid that affects trait expression is introduced without change or has been modified; if modified, methods used to modify the nucleic acid composition.

Maize with event MON 89034 was developed through Agrobacterium-mediated transformation of maize embryonic cells. The Cry1A.105 and Cry2Ab2 genes in MON89034 maize were modified before insertion to optimize expression in plants. These modifications were made to the nucleic acid sequences (not the proteins themselves) to improve transcriptional and translational efficiency in maize. The Cry1A.105 is a chimeric gene combining parts of Cry1Ab, Cry1F, and Cry1Ac, while the native Cry2Ab2 gene (from Bacillus thuringiensis) was synthetically redesigned for maize. The DNA sequence was codon-optimized for maize to enhance expression.

The modified genes were chemically synthesized in vitro using molecular biology techniques PCR based recombination. They were then inserted into a plant transformation vector (e.g., using Agrobacterium-mediated transformation). These modifications do not alter the function of the Cry proteins which still target insect pests like the European corn borer and fall armyworm, but they ensure efficient production in maize.

Method of genetic modification to generate maize with event MON 89034

MON 89034, a genetically modified corn variety, was developed using Agrobacterium-mediated transformation of the maize cultivar LH172. This method involved inserting two Bacillus thuringiensis insecticidal proteins, Cry1A.105 and Cry2Ab2, into the corn genome through the plasmid vector PV-ZMIR245. This vector contains two T-DNAs: T-DNA I, which carries the Cry gene expression cassettes, and T-DNA II, which contains the nptII gene cassette for resistance to the antibiotic kanamycin.

The process began with the transformation of immature maize embryos (Ishida et al. 1996) using the Agrobacterium tumefaciens strain ABI, which was virulent after being induced with acetosyringone. This strain, with a disarmed Ti plasmid, introduced T-DNA into the maize genome. The embryos were cultured to allow transformation, followed by selection using carbenicillin to eliminate Agrobacterium and paromomycin to select transformed cells. These cells were sub-cultured and regenerated into plants using protocols from Armstrong and Phillips (1988).

During breeding, plants that contained only the Cry1A.105 and Cry2Ab2 genes (without the nptII gene) were selected through molecular analysis, ensuring that the final MON 89034 corn was marker-free. The absence of the nptII gene was further confirmed through Southern blot and ELISA analyses. The end result was a genetically modified corn with enhanced protection against lepidopteran pests, such as corn borers, without the presence of antibiotic resistance markers.

5(3). Nucleic acid sequence of*, inter alia*, the target gene, promoter, terminator and selectable marker;

The gene elements in event MON 89034 include:

T-DNA I

The *Cry1A.105* Coding Sequence and *Cry1A.105* Protein

The *Cry1A.105* coding sequence encodes the 133 kDa *Cry1A.105* insecticidal protein that provides protection against feeding damage by lepidopteran insect pests. The *Cry1A.105* is a modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins of 90.0%, 93.6% and 76.7%, respectively. The deduced amino acid sequence of the *Cry1A.105* protein produced in MON 89034 is presented as follows;

```
  1 MDNNPNINEC IPYNCLSNPE VEVLGGERIE TGYTPIDISL SLTQFLLSEF
 51 VPGAGFVLGL VDIIWGIFGP SQWDAFLVQI EQLINQRIEE FARNQAISRL
101 EGLSNLYQIY AESFREWEAD PTNPALREEM RIQFNDMNSA LTTAIPLFAV
151 QNYQVPLLSV YVQAANLHLS VLRDVSVFGQ RWGFDAATIN SRYNDLTRLI
201 GNYTDHAVRW YNTGLERVWG PDSRDWIRYN QFRRELTLTV LDIVSLFPNY
251 DSRTYPIRTV SQLTREIYTN PVLENFDGSF RGSAQGIEGS IRSPHLMDIL
301 NSITIYTDAH RGEYYWSGHQ IMASPVGFSG PEFTFPLYGT MGNAAPQQRI
351 VAQLGQGVYR TLSSTLYRRP FNIGINNQQL SVLDGTEFAY GTSSNLPSAV
401 YRKSGTVDSL DEIPPQNNNV PPRQGFSHRL SHVSMFRSGF SNSSVSIIRA
451 PMFSWIHRSA EFNNIIASDS ITQIPLVKAH TLQSGTTVVR GPGFTGGDIL
501 RRTSGGPFAY TIVNINGQLP QRYRARIRYA STTNLRIYVT VAGERIFAGQ
551 FNKTMDTGDP LTFQSFSYAT INTAFTFPMS QSSFTVGADT FSSGNEVYID
601 RFELIPVTAT LEAEYNLERA QKAVNALFTS TNQLGLKTNV TDYHIDQVSN
651 LVTYLSDEFC LDEKRELSEK VKHAKRLSDE RNLLQDSNFK DINRQPERGW
701 GGSTGITIQG GDDVFKENYV TLSGTFDECY PTYLYQKIDE SKLKAFTRYQ
```

751 LRGYIEDSQD LEIYSIRYNA KHETVNVPGT GSLWPLSAQS PIGKCGEPNR
801 CAPHLEWNPD LDCSCRDGEK CAHHSHHFSL DIDVGCTDLN EDLGVWVIFK
851 IKTQDGHARL GNLEFLEEKP LVGEALARVK RAEKKWRDKR EKLEWETNIV
901 YKEAKESVDA LFVNSQYDQL QADTNIAMIH AADKRVHSIR EAYLPELSVI
951 PGVNAAIFEE LEGRIFTAFS LYDARNVIKN GDFNNGLSCW NVKGHVDVEE
1001 QNNQRSVLVV PEWEAEVSQE VRVCPGRGYI LRVTAYKEGY GEGCVTIHEI
1051 ENNTDELKFS NCVEEEIYPN NTVTCNDYTV NQEEYGGAYT SRNRGYNEAP
1101 SVPADYASVY EEKSYTDGRR ENPCEFNRGY RDYTPLPVGY VTKELEYFPE
1151 TDKVWIEIGE TEGTFIVDSV ELLLMEE

The *Cry1A.105* Regulatory Sequences

The expression cassette for the coding sequence of the *Cry1A.105* protein consists of the promoter (P-*e35S*) and leader for the cauliflower mosaic virus (CaMV) 35S RNA (Odell et al., 1985) with a duplicated enhancer region (Kay et al., 1987). The cassette also contains the 5' untranslated leader of the wheat chlorophyll a/b/ binding protein (L-*Cab*) (Lamppa et al., 1985), the intron from the rice actin gene (*I-Ract1*) (McElroy et al., 1991), the *Cry1A.105* coding sequence that was optimized for expression in monocots, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (T-*Hsp17*), which terminates transcription and provides the signal for mRNA polyadenylation (McElwain and Spiker, 1989).

The *Cry2Ab2* Coding Sequence and *Cry2Ab2* Protein

The *Cry2Ab2* protein present in MON 89034 is a member of the Cry2Ab class of proteins that share >95% amino sequence homology (Crickmore et al., 1998). It is a variant of the wild-type *Cry2Ab2* protein isolated from *Bacillus thuringiensis* subsp. *kurstaki*. The deduced amino acid sequence of the *Cry2Ab2* protein, together with the chloroplast transit peptide (CTP), produced in MON 89034 is as follows;

001 MAPTVMMASS ATAVAPFQGL KSTASLPVAR RSSRSLGNVS NGGRIRCMQV WPAYGNKKFE
061 IRTLSYLPPL STGGRCMQAM DNSVLNSGRT TICDAYNVAA HDPFSFQHKS LDTVQKEWTE
121 WKKNNHSLYL DPIVGTVASF LLKKVGSLVG KRILSELRNL IFPSGSTNLM QDILRETEKF
181 LNQRLNTDTL ARVNAELTGL QANVEEFNRQ VDNFLNPNRN AVPLSITSSV NTMQQLFLNR
241 LPQFQMQGYQ LLLLPLFAQA ANLHLSFIRD VILNADEWGI SAATLRTYRD YLKNYTRDYS
301 NYCINTYQSA FKGLNTRLHD MLEFRTYMFL NVFEYVSIWS LFKYQSLLVS SGANLYASGS
361 GPQQTQSFTS QDWPFLYSLF QVNSNYVLNG FSGARLSNTF PNIVGLPGST TTHALLAARV
421 NYSGGISSGD IGASPFNQNF NCSTFLPPLL TPFVRSWLDS GSDREGVATV TNWQTESFET
481 TLGLRSGAFT ARGNSNYFPD YFIRNISGVP LVVRNEDLRR PLHYNEIRNI ASPSGTPGGA
521 RAYMVSVHNR KNNIHAVHEN GSMIHLAPND YTGFTISPIH ATQVNNQTRT FISEKFGNQG
681 DSLRFEQNNT TARYTLRGNG NSYNLYLRVS SIGNSTIRVT INGRVYTATN VNTTTNNDGV
701 NDNGARFSDI NIGNVVASSN SDVPLDINVT LNSGTQFDLM NIMLVPTNIS PLY

The *Cry2Ab2* Regulatory Sequences

The *Cry2Ab2* gene expression cassette that produces the *Cry2Ab2* protein consists of the 35S promoter from figwort mosaic virus (P-*FMV*) (Rogers, 2000), the first intron from the corn heat shock protein 70 gene (I-*Hsp* 70) (Brown and Santino, 1995). The cassette also contains a *Cry2Ab2* coding sequence with a modified codon usage (CS-*Cry2Ab2*) (Widner and Whitely, 1989; Donovan, 1991) fused to a chloroplast transit peptide region of corn ribulose 1,5-biphosphate carboxylase small subunit including the first intron (TS*SSU-CTP*) (Matsuoka et al.,

1987.) The 3' non-translated region of the nopaline synthase (T-*nos*) coding region from *Agrobacterium tumefaciens* T-DNA terminates transcription and directs polyadenylation (Bevan et al., 1983).

T-DNA Borders

Plasmid vector PV-ZMIR245 contains sequences that are necessary for transfer of T-DNA into the plant cell. These sequences are termed as the Right and Left Border regions. The Right and Left Border regions each contains a border sequence that is a 2426 bp sequence that defines the extent of the DNA that should be transferred into the plant genome. They flank both T-DNA I and T-DNA II, allowing for independent transfer and integration of each T-DNA into the plant genome during transformation. The Right Borders present in PV-ZMIR245 are made of a 24 bp nucleotide sequence that was originally derived from plasmid pTiT37 which was isolated from *A. tumefaciens* (Depicker et al., 1982). The Left Borders present in PV-ZMIR245 are made of a 25 bp nucleotide sequence from the *A. tumefaciens* plasmid pTi5955, a derivative of plasmid pTiA6 (Barker et al., 1983).

T-DNA II

The *nptII* gene and NPTII protein

The *nptII* gene encodes the neomycin phosphotransferase II enzyme (NPTII) that inactivates certain aminoglycoside antibiotics such as kanamycin, neomycin and paromomycin. The use of selectable marker genes, such as *nptII*, is essential to select transformed cells under selective growth conditions. In the presence of paramomycin, cells transformed with *nptII* survive, while those that do not contain *nptII* are eliminated due to the action of the paromomycin. The T-DNA II, and therefore the *nptII* gene, is segregated out at the F1 generation.

The *nptII* regulatory sequences

The *nptII* gene cassette that produces the NPTII protein consists of the promoter (P-*e35S*) from the cauliflower mosaic virus (CaMV) 35S RNA (Odell et al., 1985). The cassette also contains the coding sequence for the NPTII protein (Beck et al., 1982), followed by the 3' non-translated region of the nopaline synthase (T-*nos*) sequence from *A. tumefaciens* which terminates the transcription and directs polyadenylation (Bevan et al., 1983).

T-DNA borders

The two T-DNA borders include; the left border, DNA region from *Agrobacterium tumefaciens* containing the 25 pb left border sequence used for transfer of the T-DNA (Barker et al., 1983) and the right border, DNA region from *Agrobacterium tumefaciens* containing the 24 bp right border sequence used for transfer of the T-DNA (Depicker et al., 1982)

Genetic Elements outside the T-DNA Borders

The backbone region outside of the inserted DNA contains two origins of replication which are necessary for replication and maintenance of the plasmid in bacteria, as well as a bacterial selectable marker gene, *aadA*, which encodes an aminoglycoside-modifying enzyme that provides resistance to the action of the antibiotics spectinomycin and streptomycin.

5(4). Description of the trait that it expresses

MON 89034 produces *Cry1A.105* and *Cry2Ab2* proteins, both of which are active against lepidopteran insect pests. MON 89034 will serve maize growers' need for controlling a wider spectrum of lepidopteran pests and help assure the durability of Bt maize. Control of these insects provided by MON 89034 is comparable to MON 810. However, MON 89034 provides a high-level control of fall armyworm (FAW) throughout the season, whereas MON 810 principally controls fall armyworm larvae during vegetative growth stage. In addition to the wider spectrum of insect control, the combination of the *Cry1A.105* and *Cry2Ab2* insecticidal proteins in a single plant, MON 89034, provides a much more effective insect resistance management (IRM) tool. Comparative biophysical studies indicate that the *Cry1A.105* and *Cry2Ab2* proteins have important differences in their mode of action, specifically in the way in which they bind to the lepidopteran midgut. Therefore, the probability of cross-resistance between these two proteins is low. Furthermore, *in vitro* and *in planta* studies with *Cry1A.105* and *Cry2Ab2* demonstrate that both proteins are highly active against the primary lepidopteran pests of corn including FAW, achieving close to or greater than the critical 95% level of control in all cases.

5(5). Methods used to construct the expression cassette;

MON 89034 was developed through Agrobacterium-mediated transformation of corn using the binary plasmid vector, PV-ZMIR245. This transformation process integrates exogenous DNA into the plant's genome (Ishida et al., 1996). PV-ZMIR245 contains two T-DNAs: T-DNA I with cry1A.105 and cry2Ab2 expression cassettes and T-DNA II with the nptII expression cassette, which confers antibiotic resistance. The expression cassette for Cry1A.105 consists of the CaMV 35S promoter, wheat chlorophyll binding protein leader, and other elements including the rice actin intron (Odell et al., 1985; Kay et al., 1987; Lamppa et al., 1985; McElroy et al., 1991; McElwain and Spiker, 1989). Cry2Ab2's expression cassette uses the figwort mosaic virus 35S promoter and corn heat shock protein 70 intron. It also includes a modified codon usage for the cry2Ab2 gene and a chloroplast transit peptide region. Both cassettes are constructed using binary plasmid vector, PV-ZMIR245 (Rogers, 2000; Brown and Santino, 1995; Widner and Whitely, 1989; Donovan, 1991; Matsuoka et al., 1987; Bevan et al., 1983). MON 89034 contains a single copy of T-DNA I (~9.3 kb) inserted at a single locus, with both cry1A.105 and cry2Ab2 genes. There are no additional plasmid sequences, except those shared with T-DNA I, confirming that the insert only encodes the two proteins, Cry1A.105 and Cry2Ab2, with no additional genetic elements.

5(6). Genetic markers;

The NptII protein functions as a selectable marker in the initial laboratory stages of plant cell selection following transformation (De Block et al., 1984; Horsch et al., 1984). The NptII enzyme uses ATP to phosphorylate neomycin and related aminoglycoside antibiotics, thereby inactivating them. Cells that produce the NptII enzyme selectively survive exposure to these aminoglycosides. The nptII coding sequence is derived from the prokaryotic E. coli transposon Tn5 (Beck et al., 1982). The purpose of inserting the gene encoding the NptII protein into maize cells along with CspB was to have an effective method for selecting cells after transformation. In general, the efficiency of plant cells transformation is often low, ranging from 1×10^{-5} to 1×10^{-4} of cells

treated (Fraley et al., 1983). Therefore, the selectable marker, NptII, was used to facilitate the screening process.

5(7). Description of identification and detection techniques of the nucleic acids;

Description of identification of the nucleic acids

The nucleic acids associated with maize with events MON 89034 including their sequences, target genes, promoters, terminators and selectable markers are described in section 5(3).

Detection techniques of the nucleic acids

The detection of Cry1A.105 and Cry2Ab2 proteins in maize event MON 89034 can be achieved using several molecular techniques. Southern blotting detects the presence of the inserted genetic sequences by analyzing DNA fragments. PCR amplifies specific DNA regions to confirm the presence of the transgenes. ELISA, on the other hand, detects the actual expressed proteins by using antibodies specific to Cry1A.105 and Cry2Ab2. These methods complement each other, with Southern blot and PCR focusing on genetic detection and ELISA measuring protein expression, ensuring accurate identification and monitoring of genetically modified maize.

5(8). Quantitative expression of sensitivity, reliability and specificity of the identification and detection techniques;

The Cry1AB.105 and Cry2AB2 proteins, expressed in Monsanto's genetically modified (GM) corn variety MON 89034, offer resistance to pests like the European corn borer and fall armyworm. Various molecular techniques are used to detect these proteins and their corresponding genes, each with varying sensitivity, specificity, and reliability. PCR-based methods, including conventional and real-time PCR, are highly sensitive for gene detection, with qPCR offering superior quantification capabilities. ELISA, a protein-based method, is commonly used for protein detection but is less sensitive than PCR. Southern and Western blotting are reliable but labor-intensive techniques, useful for confirming transgene presence and protein analysis. Next-generation sequencing (NGS) provides highly accurate and sensitive genetic analysis but is costly and complex. The choice of method depends on the specific analysis needs and available resources, with qPCR and ELISA being the most commonly used for routine testing.

5(9). Potential for nucleic acids involved in trait expression being transferred to or exchanged with other organisms;

Maize with event MON 89034, like all cultivated maize, is not sexually compatible with any wild plant species in Ethiopia. Therefore, cross-pollination can only occur between different maize cultivars. If pollen from a genetically modified maize plant fertilizes a neighboring maize crop, the traits from the genetically modified maize, including the insect pest protection traits, could potentially be transferred to the recipient crop. However, this transfer does not necessarily have negative environmental consequences, as it is unlikely to give the recipient plant a competitive advantage that would make it invasive (Sears et al., 2001).

The transfer of genes between maize crops is limited by the mobility of maize pollen, which is relatively large at 0.1 mm in diameter and typically travels only short distances. Studies show that

99% of maize pollen falls within 50 meters from the source crop, with cross-pollination practically nonexistent at distances greater than 200 meters (Pleasants et al., 2001; Sears et al., 2001; Luna et al., 2001). Factors such as the timing of flowering, pollen volume, field size, and landscape orientation also influence the likelihood of cross-pollination between maize fields (Devos et al., 2005). The risk of meaningful gene transfer and its potential environmental impact is negligible due to the limited distance over which maize pollen can travel.

In conclusion, maize with event MON 89034 does not pose a significant risk of gene transfer to wild plant species. If gene transfer occurs between maize plants, the environmental consequences are minimal. Therefore, the environmental risks associated with the cultivation of genetically modified maize for grain food, feed, and processing are negligible, with no significant impact on the ecosystem (Halsey et al., 2005; Ma et al., 2004).

5(10). Verification of the stability of the nucleic acids and factors affecting their stability

The stability of the genetically modified organism (GMO) MON 89034 over multiple generations has been demonstrated through various analyses. Southern blot analysis showed that the genetic fingerprint of MON 89034 remained consistent over seven generations, indicating stable DNA integration. T-DNA II analysis confirmed that only the intended genetic elements were integrated, with no additional modifications. Furthermore, there was no residual plasmid backbone sequence from the transformation vector. The trait for lepidopteran protection followed Mendelian inheritance patterns, supporting the stability and predictability of the genetic modification across generations.

6. Characteristics of the Vector

6(1). Nature and source of the vector;

The plasmid vector PV-ZMIR245, used for the transformation of maize cells to produce MON89034, contains 2 T-DNA. T-DNA I includes the cry1A.105 and the cry2Ab2 expression cassettes, while T-DNA II includes the nptII expression cassette (Figure 2).
Plasmid vector PV-ZMIR245 was constructed using standard molecular biology techniques. It is a binary *Agrobacterium tumefaciens* transformation vector that contains sequences that are necessary for transfer of T-DNA into the plant cell. These sequences are contained in the Right and Left Border regions which flank both T-DNA I and T-DNA II allowing for independent integration of each T-DNA into the plant genome during transformation. The T-DNA I region containing the cry1A.105 and cry2Ab2 gene expression cassettes is the portion of plasmid PV-ZMIR245 maintained in MON 89034.

6(2). Sequence of the non-coding genetic segments used in the expression cassette and to introduce it into the recipient organism to produce the modified organism;

Detail analysis of the presence or absence of the plasmid backbone is provided in **Annex 1** (Figure 1 - 3). The result confirmed that MON 89034 contains no PV-ZMIR245 backbone elements.

7. Characteristics of the Modified Organism

7(1). Information relating to the modification

a) Methods used to introduce the inserts into the recipient organism;

Maize with event MON 89034 had been developed through Agrobacterium-mediated transformation of maize embryonic cells (Armstrong and Phillips, 1988).

Method of genetic modification to generate maize with event, MON 89034

MON 89034 was developed through Agrobacterium-mediated transformation of the corn LH172, (LH172 is companies maize cultivar used for the development of the MON89034), to express the *Bacillus thuringiensis* insecticidal proteins *Cry1A.105* and *Cry2Ab2* using the plasmid vector PV-ZMIR245. Agrobacterium-mediated transformation is a well-documented process for the transfer and integration of exogenous DNA into a plant's nuclear chromosome. MON 89034 was produced using the 2T-DNA transformation plasmid vector PV-ZMIR245 which contains two separate T-DNA's. The first T-DNA, designated as T-DNA I, contains the *Cry1A.105* and the *Cry2Ab2* expression cassettes. The second T-DNA, designated as T-DNA II, contains the nptII expression cassette. During transformation, both T-DNAs were inserted into the genome. Traditional breeding was then used to isolate plants that only contain the T-DNA I (*Cry1A.105* and the *Cry2Ab2* expression cassettes) and do not contain the T-DNA II (nptII expression cassette). This resulted in the production of marker-free, lepidopteran protected corn – MON 89034.

Freshly isolated immature maize embryos were used in the transformation (Ishida et al. 1996; Rout and Armstrong, 1997). *Agrobacterium tumefaciens* strain ABI, containing plasmid PV-ZMIR245 was induced to be virulent using acetosyringone. Strain ABI also contains a disarmed Ti plasmid that is incapable of inducing tumor formation because of the deletion of the phytohormone genes originally present in the Agrobacterium *Ti* plasmid. Following inoculation with Agrobacterium, the immature embryos were transferred to a co-culture medium for one to three days to ensure transformation of individual cells. This process of Agrobacterium-mediated transformation of maize involves the attachment of the bacterium to the maize cells, which leads to transfer of the region of DNA between the Left and Right Borders of the binary plasmid (i.e., the T-DNA) into the maize genomic DNA. Each T-DNA was integrated into the plant genome at separate loci.

Following the incubation period on the co-culture medium, the immature embryos were transferred to selection medium containing carbenicillin to eliminate Agrobacterium, and paromomycin to eliminate cells that were not transformed, so that only cells containing T-DNA II and/or T-DNA I + T-DNA II survived. The resulting transformed cells were then sub-cultured several times on a selection medium and regenerated into plants according to the protocol described by Armstrong and Phillips (1988).

During subsequent breeding at the F1 generation the unlinked insertions of T-DNA I or TDNA II were segregated. The plants containing only the insert that contains the *Cry1A.105* and *Cry2Ab2* gene cassettes were selected using molecular analysis, while the plants containing the nptII cassette (T-DNA II) were eliminated from subsequent breeding. The absence of the nptII gene and the nptII protein was further confirmed by both Southern blot and ELISA analyses.

b) **Copy number of the insert;**

Insert and copy number were evaluated using Southern blot. The insert number was evaluated by digesting DNA of MON 89034 with a restriction enzyme that does not cleave within the inserted DNA. The restriction enzyme releases a restriction fragment containing the insert DNA and adjacent genomic DNA. The number of restriction fragments detected indicated the number of inserts present. Multiple copies of the transformation cassette would result in more than two bands in the gel profile. **Annex 1** provides detailed information of copy number analysis including southern Blots.

c) Presence of any vector backbone including its sequence;

Southern blot analysis showed that MON 89034 contains no backbone elements. Detailed information on vector backbone analysis is provided in **Annex 1.**

d) Sequence, functional identity and location of the altered or inserted or deleted nucleic acid with particular reference to any known harmful sequence;

Bacillus thuringiensis (Bt) is a soil-dwelling bacterium belonging to the *Bacillus* genus, which includes several species of pathogenic microorganisms. However, Bt is distinguished from these pathogenic *Bacillus* species by its production of specific insecticidal proteins (Cry and Cyt proteins) and its non-pathogenic nature to humans and animals. The Cry proteins produced by Bt (Cry1, Cry2, etc.) are highly toxic to certain insect species but do not exhibit toxicity to mammals or other non-target organisms. These proteins are specific to insect gut receptors and are not homologous to any proteins produced by known pathogenic bacteria that would cause disease in humans. The genetic sequence of Cry proteins has no significant homology with bacterial toxins known to cause illness in humans, such as botulinum toxin (produced by *Clostridium botulinum*) or cholera toxin (produced by *Vibrio cholerae*).

Cry1A.105 Gene Sequence & Functional Analysis

The origin of the sequence characteristics is the chimeric gene derived from Cry1Ab (90.0%), Cry1Ac (93.6%) and Cry1F (76.7%) from *Bacillus thuringiensis*. The functional identity to known harmful sequences revealed no homology to known toxins/allergens in humans or animals. The cry proteins are specific to insect gut receptors (alkaline pH, cadherin-like proteins), which are absent in mammals. The cry protein has no similarity to known allergens (tested via FASTA/BLAST against allergen databases, e.g., AllergenOnline). Similarly they don't have any homology to pathogenic sequences that matches to bacterial/viral virulence factors (per NCBI BLAST against pathogen databases.

Cry2Ab2 Gene Sequence & Functional Analysis:

The origin of the sequence characteristic is the native Cry2Ab2 from Bacillus thuringiensis, but synthetically redesigned for maize. The functional identity to known harmful sequences revealed no significant matches to toxins/allergens. Cry2Ab2 is structurally distinct from mammalian toxins (e.g., no homology to ricin, botulinum, etc.) and it was validated as non-allergenic by EFSA, EPA assessments. Furthermore it was validated that there was no pathogenic motifs which matches to bacterial endotoxins (e.g., LPS) or viral/fungal virulence factors.

EPA, EFSA, and FDA evaluated these genes and confirmed that there is no sequence similarity to known allergens (per Codex Alimentarius guidelines), no unintended open reading frames (ORFs) that could produce harmful peptides, and they have stable integration without disruption of endogenous toxic/allergenic maize genes. Cry1A.105 and Cry2Ab2 are artificially optimized for plant expression but retain their original insecticidal function. No credible evidence suggests homology to harmful (toxic/allergenic/pathogenic) sequences. Extensive regulatory reviews support their safety in food/feed.

7(2). Information on the modified organism:

a) Unique identifier of the transformation event;

Common	Maize or Corn
Scientific	*Zea mays* L.
Commercial	TELA maize
Transformation event Code	MON 89034
Identification Code registered with the Information Exchange Mechanism	Unique identifier is MON-89034-3
Electronic address of the Information Exchange Mechanism	https://bch.cbd.int

b) Description of phenotypic characteristics and in particular any new traits and characteristics which may be expressed or may no longer be expressed;

The general mechanism of insecticidal activity of the cry proteins is well understood. A generalized mode of action of *Cry1* proteins includes the following steps: ingestion of the protoxin crystal by the insect, solubilization of the crystal in the insect midgut, proteolytic processing of the released cry protein by digestive enzymes to produce an active toxin termed delta-endotoxin, binding of the endotoxin to receptors on the surface of mid-gut epithelial cells of target organisms, formation of membrane ion channels or pores, and consequent disruption of cellular homeostasis (English, 1992). Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die (Sacchi et al., 1986).
It has been established that this *Cry1* proteins exhibit selective toxicity towards certain lepidopteran pests, but not against other insect orders. This specificity is consistent with findings reported in the published literature, that demonstrate that proteins of the *Cry1* class exhibit lepidopteran selective toxicity (Aronson and Shai, 2001; Crickmore et al., 2005; Dulmage, 1981; Klausner, 1984; MacIntosh et al., 1990; Mendelsohn et al., 2003; Romeis et al., 2006; Whiteley and Schnepf, 1986). Selectivity based on the mode of action is a key factor in the safety of cry proteins for non-target organisms such as fish, birds, mammals and non-target invertebrates. No receptors for these proteins have been identified in the intestinal cells of mammals to date (Emmerling et al., 2001; Garner et al., 1999; Noteborn and Kuiper, 1994; Sacchi et al., 1986; Van Mellaert et al, 1988).

MON89034 is a Bt maize engineered for insect resistance, primarily against lepidopteran pests (e.g., European corn borer, fall armyworm). Its modified phenotype is primarily related to insect resistance, with no major changes in agronomic or nutritional traits compared to conventional

maize. The only new traits expressed in MON89034 are the Cry1A.105 and Cry2Ab2 proteins, which are toxic to certain insect larvae of lepidopteran pests

Regarding the phenotypic change there is reduced leaf/stem damage from corn borers and other target pests as compared with the conventional maize. Furthermore lower mycotoxin contamination due to fewer insect entry points for fungi like Fusarium on MON89034 maize. Otherwise, no significant changes in other agronomic traits such growth rate; plant height, flowering time, and yield as compared to the non-GM maize under pest-free conditions.

There is no intentional suppression of endogenous maize genes was reported and hence there is no trait that may no longer be expressed. MON89034's primary phenotypic change is insect resistance (via Cry1A.105/Cry2Ab2).

c) Stability of the traits;

The stability of the integrated DNA was demonstrated by the fact that the Southern blot fingerprint of MON 89034 was maintained in the tested generations of the breeding history. Additionally, T-DNA II analysis of multiple generations from the MON 89034 breeding history indicated that there were no T-DNA II elements present other than those (i.e., *35S* promoter, *nos* 3´ end sequence, Left Border sequence) which are common to TDNA I. Furthermore, these generations have been shown not to contain any backbone sequence from plasmid PV-ZMIR245. This stability was further confirmed by the fact that the inheritance of the lepidopteran protection trait in MON 89034 follows Mendelian segregation principle.

d) Expression of the introduced new genetic material and methods and sensitivity of measurement

The expression levels of Cry1A.105 and Cry2Ab2 proteins in MON 89034 tissues were evaluated across multiple U.S. corn production sites. The study found that both proteins were present in all tested tissues, which included leaf, root, forage, silk, pollen, grain, and stover. The average protein concentrations of Cry1A.105 ranged from 5.9 µg/g in grain to 520 µg/g in leaves at the V2-V4 stage, while Cry2Ab2 protein levels varied from 1.3 µg/g in grain to 180 µg/g in leaves at the V2-V4 stage.

For measuring protein expression, the study employed the ELISA technique, a well-established biochemical method commonly used in immunology to detect antigens or antibodies. This technique combines antibodies' specificity with the sensitivity of enzyme assays, enabling precise measurement of protein concentrations. A specific ELISA method developed and validated by Bayer was used to quantify the Cry1A.105 and Cry2Ab2 proteins in the MON 89034 plant samples.

e) Function of the expressed protein;

MON 89034 is a genetically modified crop that expresses Cry1A.105 and Cry2Ab2 proteins, which provide resistance to damage caused by certain lepidopteran insect pests. These proteins are part of the Cry protein family, whose insecticidal activity is well understood. The general mode of action for Cry1Ab protein involves several steps. First, the insect ingests the protoxin crystal. Then, in the insect's midgut, the crystal is solubilized, and the Cry protein is processed by digestive enzymes into an active toxin called delta-endotoxin. The delta-endotoxin binds to specific receptors on the surface of midgut epithelial cells in the target insect. This binding leads to the

formation of membrane ion channels or pores. These disruptions in the cell membrane result in the breakdown of cellular homeostasis, causing electrolyte imbalance and changes in pH, which ultimately paralyze the insect's gut. As a result, the insect stops feeding and dies (English, 1992; Sacchi et al., 1986).

f) Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques of the modified organism;

The quantitative expression of sensitivity, reliability, and specificity in the context of identification and detection techniques for the presence of *Cry1AB.105* and *Cry2AB2* proteins in MON 89034 (a genetically modified maize variety) can be defined and evaluated through various several molecular techniques, including **ELISA** (Enzyme-Linked Immunosorbent Assay) and other molecular techniques such as **qPCR** (quantitative Polymerase Chain Reaction) and **Western blot**. In particular ELISA is suitable for screening large numbers of samples. It can detect Cry proteins at low concentrations, making it useful for detecting trait expression in small quantities. Furthermore, the use of specific antibodies tailored to the Cry proteins ensures reliable identification and quantification of the target proteins.

g) History of previous releases or uses of the modified organism;

The use of MON 89034 began with regulatory approval for commercial cultivation in several countries, starting with the United States in 2008. It was developed as a tool for integrated pest management in corn farming, aiming to reduce the need for chemical insecticides. Over time, MON 89034 gained approval for import and cultivation in multiple regions, including Canada, Mexico, and parts of the European Union, for specific uses in animal feed and food processing. In Africa, Nigeria and South Africa are the two leading countries in commercializing MON 89034. Recently, transgenic maize expressing Cry1A.105 and Cry2Ab2 proteins has been imported and tested under CFT and contained environments in Kenya, Ethiopia, Mozambique and Rwanda with good trait performance being realized (AATF, 2021).

The history of MON 89034 also involved ongoing monitoring and research to assess its environmental and health safety. It underwent extensive evaluations by various regulatory bodies, including the U.S. Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the European Food Safety Authority (EFSA). These evaluations confirmed the safety of MON 89034 for human consumption, animal feed, and environmental impact, leading to its widespread adoption.

h) Risks to human or animal health, biological diversity and the environment;

Comparative studies of the genetically modified maize variety MON 89034 and its conventional counterparts have confirmed their compositional equivalence, with the only difference being the introduction of specific traits (Cannon, 1993; EPA, 1988; IPCS, 1999; Sjoblad et al., 1992; De Schrijver et al., 2007; EFSA, 2007). These assessments suggest that MON 89034 is compositionally similar to conventional maize, with the exception of the traits it has been engineered to express. These traits are designed to offer agronomic benefits, such as insect resistance, without affecting the maize's overall composition or processing characteristics (De Schrijver et al., 2007).

Comprehensive safety evaluations have confirmed the safety of MON 89034 in terms of human and animal consumption, as well as its environmental impact, supporting its regulatory approval for commercial cultivation in various countries (CBD, 2019; James, 2018). These evaluations included a detailed molecular analysis of the introduced DNA, an assessment of the Cry1Ab protein expressed by the plant, a compositional analysis of the maize grain and forage, and an environmental impact review (Sanders et al., 1998). The results indicate that MON 89034 is safe for human consumption, animal feed, non-target organisms, and beneficial insects, and that its grain and forage are as nutritious and safe as those from conventional maize varieties.

i) Generation time in natural ecosystems, sexual and asexual reproductive cycles;

Reproductive Biology of Maize: The genetic modification of maize with MON 89034 does not affect its reproductive biology. The only introduced trait is insect protection, which does not alter typical reproductive characteristics. Therefore, maize with MON 89034 is expected to have the same reproductive ability as conventional maize.

Stability of Integrated DNA: The stability of the integrated DNA in MON 89034 was confirmed through Southern blot analysis, which showed consistent fingerprinting across generations. T-DNA II analysis also indicated no extra T-DNA elements beyond those expected, and no backbone sequence from the plasmid PV-ZMIR245 was detected.

Mendelian Inheritance: The inheritance of the insect protection trait in MON 89034 follows Mendelian principles of segregation, further confirming the stability and consistent transmission of the modified trait across generations.

j) Information on survival, including seasonality and the ability to form seeds, spores, sclerotia and other survival structures;

The genetically modified maize MON 89034, developed to resist certain pests, has survival characteristics linked to its ability to endure environmental conditions. This maize has been engineered for pest resistance, primarily targeting the maize stem borer and fall armyworm, allowing it to grow and reproduce under varying levels of pest pressure. Regarding survival in the environment, MON 89034's modified traits do not specifically alter its ability to endure seasonal changes. Like conventional maize, it completes its life cycle within one growing season, typically surviving through germination, flowering, and seed production.

MON 89034 does not produce spores, sclerotia, or other specialized survival structures that are typically associated with fungi or other organisms capable of long-term survival through environmental stress. Instead, its survival mechanism relies on the generation of seeds, which are the primary method of propagation. The seeds of MON 89034 carry the genetic modifications and, under appropriate conditions, can grow into plants exhibiting pest resistance. These seeds are seasonal, meaning they are produced annually and depend on suitable growing conditions for germination, but there are no additional survival forms such as spores that allow the maize to endure outside of the growing season.

k) Pathogenicity, infectivity, toxicity, virulence, allergenicity, ability to be a carrier (vector) of pathogens,

[Identity of possible vectors to transmit it to humans or animals as a disease causing agent, host range, possible activation of latent viruses, ability to colonize other organisms, ability of nucleic

acids or other molecules that affect trait expression to be introduced into the cells or tissues of other species;]

Pathogenicity: Modified maize MON 89034, which expresses the Cry1Ab, Cry1Ac, Cry2Ab2, and Cry2Ae insecticidal proteins, is not pathogenic to humans, animals, or plants. These proteins are engineered to target specific insect pests, such as maize stem borer and fall armyworm, but they do not cause disease in humans or other non-target organisms. Studies have shown that these genetically modified organisms (GMOs) do not pose a risk of pathogenicity to the environment or human health (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

Infectivity: MON 89034 maize does not possess any infectious characteristics. The Cry proteins produced by this genetically modified maize are not capable of infecting or replicating in humans, animals, or microorganisms. These proteins are specific to certain insect pests and do not have the ability to cause infections in non-target organisms. Furthermore, MON 89034 maize does not carry any infectious agents such as viruses or bacteria that would contribute to its ability to infect other organisms.

Toxicity: The Cry1Ab, Cry1Ac, Cry2Ab2, and Cry2Ae proteins in MON 89034 maize are designed to be toxic to specific insect pests, but they are not toxic to humans, animals, or other non-target organisms. Toxicity studies have shown that these proteins are degraded in the gastrointestinal tract of mammals and do not pose a toxic risk. The proteins exhibit selective toxicity, targeting only certain insect species, and have no harmful effects on humans or animals when consumed.

Virulence: The modified maize MON 89034 does not demonstrate virulence. Virulence refers to the ability of an organism to cause disease, and since the Cry proteins are insecticidal, they only affect certain pests, not humans or other animals. The maize does not have any properties that would make it virulent to any organisms other than the specific insect pests it is designed to target. Therefore, it does not present a risk of virulence to humans, animals, or other non-target species.

Allergenicity: MON 89034 maize has been evaluated for potential allergenicity, and the Cry proteins expressed in the plant are not known to be allergens. In studies, the Cry proteins have not caused allergic reactions in humans or animals, and the genetic modification does not introduce new allergens into the maize. The modified maize is considered to have a similar allergenic profile to conventional maize, with no significant increase in the risk of allergic reactions.

Ability to be a Carrier (Vector) of Pathogens: MON 89034 maize does not have the ability to act as a vector or carrier of pathogens. The genetic modifications made to the maize do not alter its ability to interact with pathogens, and it does not serve as a vector for diseases or pests. The Cry proteins do not have the potential to facilitate the transmission of pathogens, and the maize itself does not carry any viruses, bacteria, or other microorganisms that could spread diseases to other plants or animals.

The information provided above is derived from various studies and regulatory assessments, including those conducted by organizations such as the U.S. Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and other global regulatory bodies involved in GMO safety evaluation. The specific data on MON 89034 can be found in safety r eviews and the Environmental Impact Assessments for genetically modified organisms (GMOs) (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

l) Antibiotic resistance, and potential use of these antibiotics in humans or domestic animals for prophylaxis or therapy;

Maize with events MON 89034 contains Cry1A.105 and Cry2Ab2 proteins for pest resistance, as well as the NptII protein, which serves as a selectable marker during initial laboratory stages of plant cell selection after transformation. The NptII protein helps maize cells survive exposure to aminoglycoside antibiotics by inactivating them, allowing for selective survival. The NptII gene comes from the E. coli transposon Tn5 and is used to increase the efficiency of plant cell transformation, which is typically low and they do not typically persist in the final commercial product, meaning that they do not remain in the edible parts of the plant.

Concerns about Antibiotic Resistance: The broader concern with GM crops that use antibiotic resistance genes lies in the potential for these genes to be transferred to bacteria in the environment. Horizontal gene transfer could theoretically lead to the spread of resistance traits among microbial populations. However, research has found that such transfers are rare and do not pose significant risks when antibiotic resistance genes are used as part of GM crops. In the case of MON 89034, the absence of these resistance genes in the final product mitigates this concern.

Furthermore, the potential for GM crops like MON 89034 to directly affect human or animal antibiotic therapy is minimal, as the maize does not carry active antibiotic resistance genes, and the rigorous safety assessments ensure that the products of GM crops do not pose a direct risk to human health or contribute to resistance in microbial populations.

m) Involvement in environmental processes, primary production, nutrient cycling, decomposition of organic matter and other activities;

Cry proteins, commonly used in Bt crops, are highly specific in targeting insects without posing significant risks to non-insect animals. Extensive studies have shown that Cry proteins, such as Cry1A.105 and Cry2Ab2, do not harm mammals, birds, aquatic animals, soil organisms, or most non-target insects. Tests on species like earthworms and beneficial insects have confirmed their safety, with no adverse effects observed for endangered species or non-insect organisms in terrestrial or aquatic environments (Federici, 2002; Romeis et al., 2006; Mendelsohn et al., 2003). This high specificity suggests that Cry proteins have a minimal environmental impact when used in agriculture. In addition to their limited impact on non-target species, Cry proteins in the soil degrade relatively quickly, influenced by soil pH and clay content. Research has shown that in neutral or near-neutral pH soils, Cry proteins degrade efficiently, with no significant accumulation in long-term plantings of Bt crops (Dubelman et al., 2005; Ahmad et al., 2005). Laboratory studies report rapid dissipation of Cry proteins, with the DT50 and DT90 times ranging from 0.5 to 7 days and 3 to 19 days, respectively (Wright et al., 2004). Studies on soil organisms, including microflora and fauna, found no significant negative effects, indicating that Cry proteins do not disrupt soil ecosystems (Donegan et al., 1996; Al-Deeb et al., 2003; Koskella and Stotzky, 2002). Over all, these results suggest that the risk to soil-dwelling organisms from their exposure to MON 89034 which expresses Cry1A.105 and Cry2Ab2 proteins are negligible.

l. Risk of passing modification to other organisms;
i) Characteristics of the modified maize, which may cause an adverse effect

Maize with event MON 89034 is not compatible for sexual reproduction with wild plant species in Ethiopia, limiting genetic transfer to other plants. The potential for cross-pollination exists, where maize pollen can be carried by the wind to fertilize neighboring maize crops. If this occurs, the introduced traits in MON 89034 maize could be passed on, which could affect the recipient maize. However, this potential outcrossing is considered a characteristic of GM plants that may cause environmental effects.

ii) Potential consequences of the adverse effect, if it occurs

Outcrossing itself is not inherently harmful. However, if the transferred traits (such as drought tolerance or insect pest protection) provide a competitive advantage to the recipient plant, it could lead to the development of an invasive species. Despite this possibility, the traits of the GM maize are unlikely to confer a meaningful competitive advantage, thus reducing the risk of any adverse effects on the environment.

iii) Likelihood of the occurrence of the potential adverse effect

The potential for gene transfer is limited to cross-pollination between maize plants. Maize pollen, though carried by the wind, typically travels only short distances, with most pollen falling within 5 meters of the crop. Studies show that over 99% of maize pollen falls within 50 meters of the source, and significant cross-pollination is unlikely beyond 100 meters. Factors such as flowering synchrony, pollen volume, and wind direction can also affect cross-pollination, but the risk of significant environmental impact is minimal.

iv) Estimation of the risk

There is no significant risk of gene transfer from MON89034 maize to wild plant species, and the likelihood of gene transfer to other maize crops is negligible as far as cross pollination limitation and biological barriers of the maize are in place (Chumakov et al., 2019). In cases where gene transfer does occur, the environmental consequences are minimal (BPPD, 2007i; BPPD, 2007j). Thus, the environmental risk associated with cultivating MON89034 maize for grain food, feed, or processing is very low (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

v) Application of management strategies

Given the negligible risk associated with MON89034 maize, no additional risk management strategies are required.

7(3). Considerations of impacts on health:

i) Toxic, allergenic or other similar effects of the modified organism on humans or animals;

The consideration of the health impacts of genetically modified maize MON 89034, which expresses the Cry1Ab, Cry1Ac, and Cry2Ab2 proteins, primarily focuses on potential toxicological, allergenic, and other similar effects on humans and animals. In evaluating its safety, numerous studies are conducted to determine whether these proteins could have harmful effects when consumed. These studies include tests for acute toxicity, chronic toxicity, and potential to cause allergic reactions. MON89034 maize has undergone extensive safety evaluations by regulatory agencies, and the available data has shown no significant risks of toxicity or allergenicity for humans or animals (Annex II; Table 1 and Table 2).

Regarding toxicological effects, various animal feeding studies have shown no evidence of adverse outcomes when MON89034 maize is included in the diet. The Cry proteins expressed by the maize are specific to insect pests and do not have harmful effects on humans or animals due to their mode of action, which involves binding to insect gut receptors that are not present in mammals. Additionally, no significant differences were found in the composition of MON89034 compared to conventional maize, further supporting its safety for human and animal consumption.
On the allergenic potential, extensive testing has shown that MON 89034 maize does not produce new allergens or display any significant potential to cause allergic reactions. The Cry proteins are not known to cause allergic responses in humans, as they are rapidly broken down in the digestive system and have no lasting effects on human or animal health. In fact, studies conducted to assess the potential for cross-reactivity with known allergens have not revealed any concerns.
Overall regulatory agencies have concluded that MON 89034 maize, including Cry1Ab.105 and Cry2Ab2 proteins, poses no unreasonable risks to human or animal health (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

History of safe use (FSANZ, 2008; EPA, 2010)

Cry1A.105

Cry1A.105 is a chimeric protein engineered from functional domains of three wild-type Bacillus thuringiensis (Bt) Cry proteins (Cry1Ab, Cry1Ac, and Cry1F). Specifically, it contains Domains I and II from Cry1Ab/Cry1Ac, Domain III from Cry1F, and the C-terminal domain from Cry1Ac. Although Cry1A.105 itself has no direct history of use, it shares 93.6% amino acid sequence similarity with Cry1Ac, a well-characterized protein with a long history of safe consumption in food (FSANZ, 2008).

Microbial pesticides containing Cry1Ac (e.g., DIPEL®) have been used in Australia and New Zealand since 1989, while a Cry1Ac/Cry1F chimeric protein (Lepinox™) has been approved in the U.S. since 1997 for lepidopteran pest control. Both Food Standards Australia New Zealand (FSANZ) and regulatory agencies have previously assessed Cry1Ac and Cry1F, finding no safety concerns related to human toxicity (FSANZ, 2008).

Cry2Ab2

The Cry2Ab2 protein in MON 89034 corn is 88% identical to the wild-type Cry2Ab2 produced by B. thuringiensis subsp. kurstaki. This protein has been used for years in microbial pesticides such as Dipel, Cutlass, and Crymax. Additionally, food from Cry2Ab2-expressing cotton (identical to the version in MON 89034) was approved in Australia and New Zealand in 2002 (Application A436), with regulators concluding that Cry2Ab2 is unlikely to be toxic to humans (FSANZ, 2008).

Both Cry1A.105 and Cry2Ab2 have well-documented histories of safe use through closely related proteins in microbial pesticides and genetically modified crops. Regulatory assessments in multiple countries support their low toxicity risk to humans, reinforcing their safety profile in MON 89034 corn (FSANZ, 2008).

Information on protein source, cry protein proportion compared to total expressed protein, bioinformatics analysis

The levels of Cry1A.105 and Cry2Ab2 in corn tissues collected from MON 89034 plants grown at five field sites in the U.S. were determined using enzyme-linked immunosorbent assays (ELISA). The means for Cry1A.105 protein levels across all sites were 5.9 µg/g dry weight (dwt) in grain, 42 µg/g dwt in forage, 12 µg/g dwt in pollen, 520 µg/g dwt in over season leaf collected at growth stage V2-V4 (OSL-1), 120 µg/g dwt in leaves OSL-4 (collected at growth stage pre-VT), 12 µg/g dwt in forage root, and 50 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 72-520 µg/g dwt in leaf, 42-79 µg/g dwt in root, and 100-380 µg/g dwt in whole plant. The means for Cry2Ab2 protein levels across all sites were 1.3 µg/g dwt in grain, 38 µg/g dwt in forage, 0.64 µg/g dwt in pollen, 180 µg/g dwt in OSL 1, 160 µg/g dwt in OSL-4, 21 µg/g dwt in forage root, and 62 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels across all sites ranged from 130-180 µg/g dwt in leaf, 26-58 µg/g dwt in root, and 39-130 µg/g dwt in whole plant.

Different techniques were used to characterize and compare the plant-produced and the E. coli-produced proteins this includes sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, densitometry, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass 12 Bacillus thuringiensis Cry1A.105, and Cry2Ab2 Protein in Corn Biopesticide Registration Action Document spectrometry, glycosylation analysis, N-terminal amino acid sequencing, and insecticidal activity assays. Furthermore, short descriptions of enzyme-linked immunosorbent assay (ELISA) methods for detecting and quantifying Cry1A.105 and Cry2Ab2 (EPA, 2010).

ii) Comparison of the modified organism with the donor and recipient or unmodified parental organisms regarding pathogenicity, allergenicity, or other similar effects in humans or animals;

As indicated above, maize with event MON 89034 has been demonstrated to be compositionally equivalent to conventional maize. Please refer 7(1) d

iii) Capacity for colonization;

The potential for maize with events MON 89034 to adversely impact the environment in Ethiopia is evaluated below, based on a step-wise risk assessment process comprising of: a) identification of potentially harmful characteristics of the GM plant; b) potential consequence

of the adverse effect (assuming fully realized); c) likelihood of the adverse effect to occur for this product and a characterization of the actual hazard potential of the modified plant characteristic; d) estimation of the risk and e) necessary risk mitigation measures, if applicable. In the previous sections of this application, maize with events MON 89034 was shown not to be different from conventional maize in its agronomic, phenotypic, compositional, nutritional and safety characteristics. This suggests that interactions of this maize with the biotic environment have not been changed compared to conventional maize. Thus, no adverse environmental effects are expected during environmental release of the maize in Ethiopia.

a) Characteristics of the genetically modified maize, which may cause adverse effect

Based on centuries of experience with conventional domesticated maize, there is no potential for maize to be invasive of natural habitats or persist in the agronomic environment without human intervention. Maize is a poor competitor, which outside of cultivation has no meaningful impact on biodiversity or the environment.

Extensive characterization of the maize (which included molecular, expression, composition, and phenotypic data) presented in this application demonstrated that the only meaningful difference between the modified maize and conventional maize is the reduced yield loss due to insect infestation conditions conferred by the introduction of the *Cry1A.105* and *Cry2Ab2* genes.

b) Potential consequences of the adverse effect, if it occurs

Increased persistence could elevate maize to weed status and could result in an invasive species, thus being persistent and spreading in the environment. However, such changes or impact would be atypical of *Zea mays*, and have not been reported through the decades of plant breeding, mutagenesis and other forms of introducing genetic diversity into maize (Martinez-Soriano et al., 2002).

c) Likelihood of the occurrence of the potential adverse effect

Maize is described as a poor competitor, with limited environmental impact outside of cultivated areas. It was originally introduced from Mexico as an annual crop, and due to centuries of domestication, it is not persistent or invasive in the natural environment. Maize requires human intervention to survive and cannot thrive as a weed. Despite its widespread cultivation, including in Ethiopia, maize is not found growing outside of cultivated agricultural fields, such as in fencerows, roadsides, or natural habitats. This lack of spread is due to factors like the absence of seed dormancy and poor survivability of seeds in soil, compounded by soil cultivation practices that destroy existing vegetation (OECD, 2003; Shaw, 1988).

Maize kernels, being its only survival structures, are not predisposed to natural dissemination due to the plant's specific floral structure. Unlike weedy plants, maize has a polystichous female inflorescence (ear) on a stiff central spike (cob) surrounded by husks. Although kernels can be disseminated through mechanical harvesting, insect or wind damage, or by wild animals, they are unlikely to establish themselves as weeds. While kernels can survive mild winters and germinate the following year, maize cannot persist as a weed (Hallauer, 1995; OECD, 2003b).

The genetically modified (GM) maize with event MON 89034, which has been engineered for drought tolerance and insect protection, does not exhibit significant differences from conventional maize varieties. Data from field trials suggest that the genetic modifications do not alter the maize's phenotypic, agronomic, reproductive, survival, or dissemination characteristics. Consequently, it is unlikely that this GM maize would be any more persistent or invasive in the environment than conventional maize varieties.

The overall conclusion is that maize with event MON 89034 is unlikely to be more weedy or invasive than conventional maize. Therefore, the likelihood of this GM maize spreading in the environment is considered negligible.

d) Potential consequences of maize with event MON 89034 was it to be weedy.

The EPA has determined that MON89034 corn poses negligible weediness risk, as there are no wild or weedy relatives of corn in the U.S. and similarly there are no documented evidences of wild relatives of maize in Ethiopia capable of receiving and expressing its transgenes. While teosintes and Tripsacum species are maize relatives, natural hybridization is extremely unlikely due to chromosomal differences (maize 2n=20 vs Tripsacum 2n=36/72), reproductive barriers, and habitat separation. Any rare hybrids show severe sterility and cannot survive temperate winters. Teosintes, the only fully interfertile relatives, are not present in Ethiopian agricultural areas, with no natural populations reported so far (EPA, 2010).

Both the EPA and USDA-APHIS agree that neither conventional nor genetically modified corn poses a weed risk. Historical evidence from Mexico, where maize and teosinte have coexisted for millennia, shows no emergence of weedy hybrids. Thus, the introduction of MON89034 does not increase the likelihood of creating invasive or hard-to-control weeds (EPA, 2010).

e) Estimation of the risk

It is concluded that, like for conventional maize, the likelihood of maize with events MON 89034 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in modified maize when compared to conventional maize. In the unlikely event of establishment of the modified maize, *e.g.* from a grain spilt in the environment, its introduced traits would have negligible consequences for the environment. Hence, the risk of unintentional spreading of maize with events MON 89034 through increased weediness of this maize is negligible.

f) Application of risk management strategies

Since the risk is negligible, risk management strategies are not considered necessary. Table 1 below summarizes the risk evaluation for the potential risk of the modified maize becoming weed.

Table 1. Summary of Risk assessment on potential for weediness, persistence and invasiveness

Characteristics of the modified plant which may cause an adverse effect	**Potential consequence of the adverse effect, if it occurs**	**Likelihood of occurrence of the potential adverse effect**	**Estimation of the risk posed by the characteristic of the modified plant**	**Risk Management Strategy y for the modified plant**
Presence of the introduced drought tolerance and insect pest protection traits	Increased persistence or invasiveness, resulting in an invasive species spreading in the environment	Negligible	Negligible	(not applicable)

***Note:** Table 1 refers the general assessment matrix of weediness, persistence and invasiveness for different events of corn. It is not referring for specific events, the assessment works for every event. Here it is possible to cancel the drought and leave the insect traits alone. Please also refer 7 (3) iii, d above.

iv) If the organism is pathogenic or toxic or allergenic to immune-competent humans or animal, the following information is required:

Maize with events MON 89034 is not pathogenic or toxic to humans or animals, hence the information list down is not applicable

1. diseases caused and mechanism of pathogenicity including invasiveness and virulence;
2. communicability;
3. infective dose;
4. host range and possibility of alteration of hosts;
5. possibility of survival outside of humans or other organisms;
6. vectors or means of dissemination;
7. biological stability;
8. availability of appropriate therapies.

PART THREE

INFORMATION RELATING TO THE RECEIVING ENVIRONMENT AND INTERACTION BETWEEN THE MODIFIED ORGANISM AND THE RECEIVING ENVIRONMENT

8. Information on the Deliberate Release

8(1). Dascription of the proposed deliberate release, including the purpose and foreseen outcome;

This application requests approval for the deliberate release and cultivation of maize with event MON 89034, which contains traits that protect against lepidopteran stem borer and Fall Armyworm (FAW) pests. The introduction of this maize would serve as an additional tool to reduce the damaging effects caused by these pests, which are significant threats to maize production. The event MON 89034 maize would be adapted to Ethiopia’s agro-ecologies, benefiting local farmers by providing access to insect-resistant seeds. This could lead to improved maize productivity and increased income, enhancing the commercial viability of maize farming in the region.

If the release is approved, the maize would include the Cry1A.105 and Cry2Ab2 genes, which confer resistance to the identified pests. The environmental release of this maize is expected to provide a sustainable solution to pest problems that have traditionally harmed yields, offering a potential boost to Ethiopia's agricultural sector. The data and evidence provided in the submission indicate that there are no identified environmental hazards or health and safety concerns associated with the maize, supporting the conclusion that maize containing event MON 89034 is unlikely to present risks to the environment. Additionally, food and feed from this maize are considered as safe as those from conventional maize varieties.

Based on the weight of the evidence provided, the submission concludes that the environmental risks associated with the release of maize with event MON 89034 are minimal. Therefore, no specific risk management strategies are deemed necessary for its cultivation in Ethiopia. The overall assessment suggests that the release of this genetically modified maize will not pose a significant risk to the environment or human health.

8(2). Planned dates and durations of the release

Once the biosafety approval is granted and successful demonstrations are completed, the maize will be ready for release. The specific date of the release will depend on the completion of these steps, but the projected timeline indicates that the maize could be commercially available or distributed to farmers sometime in 2026, potentially in the first few months of the year.

8(3). Method to be used for the release;

Maize with events MON 89034 being developed are intended for potential cultivation in all maize growing regions of Ethiopia to address challenges brought about by insect pests. The release of this genetically modified (GM) maize will follow the existing guidelines which are designed to ensure that the introduction and commercialization of GM crops are carried out in a safe manner.

8(4). Quantity of modified organism to be released;

An estimated initial batch of 180,960 seeds (approximately 80kg) will be imported and availed for variety release testing under National Performance Trials (NPTs) and for Distinctness, Uniformity and Stability (DUS) testing as well as for on-station and on-farm demonstrations. For NPT testing, this covers three replications, for single growing seasons in at least 6 sites in the maize growing ecological zones. The commercial cultivation of released hybrids will follow in 2026.

8(5). Site preparation prior to release (type and method of cultivation, mining, irrigation, or other activities);

Site preparations prior to the planting of subsequent crops (which include destruction of any existing weedy vegetation, soil cultivation, furrow preparation etc) will be undertaken. The preparation will not be different from existing types used for conventional maize in Ethiopia. The technology does not alter the site preparation aspects of the cultivation.

8(6). Information on, and results of, previous releases of the modified organism especially at different scales and in different ecosystems

As mentioned above, in section 2(g) of part 2, since 1996, a variety of biotechnology-derived crops containing Cry proteins from Bt have been commercialized and maize with event MON 89034 has been used extensively for food and feed. There exists a long history of safety for the Cry proteins, based on the extensive commercial use of MON 89034 maize on millions of hectares around the world (James 2018).

9. Survival, multiplication, dispersal and trait expression

9(1). Biological features which affect the survival, multiplication and dispersal of the modified organism;

The modified maize and conventional maize does not have any significant difference in terms the inserted gene to the modified one. Rather the presence of the cry proteins on the modified maize MON89034 provides a selective advantage to maize, particularly in environments where pest pressure is high. Otherwise, as we know maize originated in the highlands of Mexico 7,000 to 10,000 years ago (Smith, 1998). European explorers introduced maize to the rest of the world that allowed it to become an essential crop for food, feed and fuel (Goodman 1988). From its likely origin as a wild grass, maize has undergone continuous breeding, modification, and selection for properties that suit the needs of consumers and growers who wish to produce maize grain.

Maize is now grown across a wide range of ecological conditions including different soil types, altitude and rainfall. Maize is grown over a wide range of conditions because of its many divergent types that have been bred for this purpose. Most maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21 and 27° C and the frost-free season lasts 120 to 180 days. Hence, the biological features of MON 89034 maize differ from the conventional due to its genetic modification for pest resistance, otherwise all the other biological features for gene flow, survival, multiplication, and dispersal are the same.

9(2). Known or predicted environmental conditions which may affect the survival, multiplication and dispersal (wind, water, soil, temperature, pH, pollutants such as pesticides, heavy metals, horizontal gene transfer and others) of the modified organism or of its genes or other biochemical that affect trait expression.

As described above, maize is grown in a wide range of climatic conditions. Rainfall of 350 mm is approximately the lower limit for production without irrigation without any upper limit for growing maize, although excess rainfall decreases yields. It is, however, grown mainly in the moderate to high rainfall regions of Ethiopia. Maize with events MON 89034 will be grown in the same geographic areas as conventional maize.

10. Interactions with the environment

10(1). Habitat types expected to be suitable for the modified organism;

The modified maize will be grown in the same habitat that is suitable for conventional maize. As noted by OECD (2003), maize is not invasive of natural habitats, does not persist or disperse anywhere in the world without the human intervention. Early domestication and diversification through selection occurred in Meso-America. Maize is grown across a wide range of ecological conditions including soil types, altitude and rainfall. Currently, maize is grown over a wide range

of conditions because of its many divergent types that have been bred for this purpose. The bulk of the maize is produced between latitudes 30°and 55°, with relatively little grown at latitudes higher than 47° anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21°C and 27°C and the frost-free season lasts 120 to 180 days. A summer rainfall of 15 cm is approximately the lower limit for maize production without irrigation. Experience with maize imported for use as foods or feeds, or directly in processing, has demonstrated that stable populations do not establish, persist or proliferate as a result of this practice.

10(2). Experiments carried out on the behavior of the modified organism and an assessment of its ecological impact carried out in simulated natural environments, such as growth rooms and greenhouses;

This section summarizes a Technical Report presented to the Environmental Protection Authority (EPA) of Ethiopia on studies of the efficacy of insect protected (Bt) maize in confined fields from 2023–2024 at three sites.

i) Location and development of confined field sites

Two confined field trial (CFT) locations were selected and developed with state-of-the-art drip irrigation facilities covering two-hectare land area. To prevent the dissemination of the experimental plant and its subsequent introduction into the wider environment, the CFT locations are fenced, and appropriate procedures were put in place as per the requirement of the federal biosafety regulations and institutional biosafety policy. Bako and Wondogenet were selected from mid altitude high potential maize growing agroecology with altitude of 1,650 and 1780m above sea level (asl), respectively. The other CFT site was at Melkassa and was selected for its low-altitude drought stress agroecology with an altitude of 1,550 m asl.

ii) Experimental maize genotypes

Six maize test genotypes with stacked events of Bt insect protection (*Bt* gene Event MON 89034) and its respective non-GM versions (isogenic) were imported from Bayer, RSA following the import permit requirements of the Ethiopian Agricultural Authority. A total of two commercial maize hybrid varieties were used as local checks (see details of the genotypes in Table 2). The test genotypes were used for proof of concept and meant to test for the efficacy of Bt genes and traits.

Table 2. Description of Bt maize and check varieties grown under the current CFT at Melkassa, Bako and Wondogenet CFT sites, 2024.

No	Entry name	Description
1	Bt_01	Hybrid with MON 89034 event
2	Isogenic_01	Isogenic hybrid without MON 89034 event
3	Bt_02	Hybrid with MON 89034 event
4	Isogenic _02	Isogenic hybrid without MON 89034 event
5	Bt_03	Hybrid with MON 89034 event
6	Isogenic _03	Isogenic hybrid without MON 89034 event
7	Bt_04	Hybrid with MON 89034 event
8	Isogenic _04	Isogenic hybrid without MON 89034 event
9	Bt_05	Hybrid with MON 89034 event
10	Isogenic _05	Isogenic hybrid without MON 89034 event
11	Bt_06	Hybrid with MON 89034 event

12	Isogenic _06	Isogenic hybrid without MON 89034 event
13	Check	MH140 for Melkassa and Wondogenet
14	Check	BH5211 for Bako

iii) *Methodology*

Three independent CFTs were conducted across the three sites in 2023/24. The first CFTs were conducted with the objective to evaluate the efficacy of the test Bt genotypes for insect protection against stem borer and FAW pests. A total of two management conditions were applied in three locations: (a) Trail with insecticide application (Optimum Trial); (b) Trail with no insecticide application (Bt Trial). The Bt trial was conducted under natural infestation of pests. All other recommended agronomic practices remained the same as per specific requirements for maize production in the locations across the three environments.

Experimental design and net-plot size of experimental units

Randomized Complete Block Design was used for all the trials. The number of replications was varied based on the type of the trials (Optimum, and Bt) and locations. During the first season at Wondogenet and Melkassa, the number of replications for BT and Optimum were six and three, respectively. The same replication was used for the second CFT in Bako during the main rainy season. Each experimental unit at all locations consisted of 2 rows of 5 m long with spacing of 0.75 between rows and 0.25 m between plants. A total of 53,333 plant population per hectare were expected in all locations.

iv) Data collection

Data were scored for FAW and stem borer damage and the maize ears were harvested manually on plot basis. Shelling, weighing and moisture recording were done. The collected data was used to estimate grain yield (GY) and expressed in t/ha at 12.5% grain moisture using the formula bellow:

$$\text{GY (t/ha)} = \frac{\text{GW} \times (100 - \%\ \text{Moisture})/87.5}{\text{Plot area}}$$, Where, GW = grain weight.

v) Data analyses

Data collected were analyzed statistically for each site (Melkassa, Bako and Wondogenet). Pairwise comparison of Bt versus Non-Bt genotypes was carried out using a t-test.

vi) Results of confined field trials in Ethiopia

Analysis of data on MON89034 maize genotypes in the CFTs planted in Wondogenet, Melkassa and Bako in 2024, showed significant ($P < 0.05$) differences among the Bt and non-Bt genotypes for grain yield and most other traits under the study, whereas no significant difference were observed among Bt and non- Bt tested genotypes for optimum trials (optimum trial with insecticide application).

a) Evaluation of efficacy of GM insect protection trait (Bt MON89034) on maize growth, grain yield, and pests' protection in Melkassa (Low moisture dry land area)

At Melkassa, under natural target pest infestation at the CFT site, the highest relative grain yield advantage was observed with Bt that had 29.1.4% and 108.1% grain yield than their non-Bt isogenic versions and commercial check, respectively (**Table 3**). The mean leaf damage score (1 to 9 rating scale) by FAW was higher for isogenic (5.9) and check (7.3) whereas lower mean leaf damage was recorded for Bt hybrids (3.0). The lower leaf damage score indicated the effectiveness of the Bt hybrids of MON89034 event in conferring resistance against FAW and stem borer.

Under optimum-trial condition with pests controlled using chemicals, the Bt maize and their isogenic hybrids were almost similar (**Table 4**), However, they performed superiorly than the commercial checks in grain yield and other traits under the study with three-time chemical application at a rate 120ml/ha over the commercial cheeks. The result indicated that the effectiveness of the Bt MON89034 gene is similar with effective chemicals in conferring for control the two distractive insects such as FAW and stem borer. However, the Bt gene gave more advantages working friendly with the environment and reducing human hazard than using chemicals for insect protection.

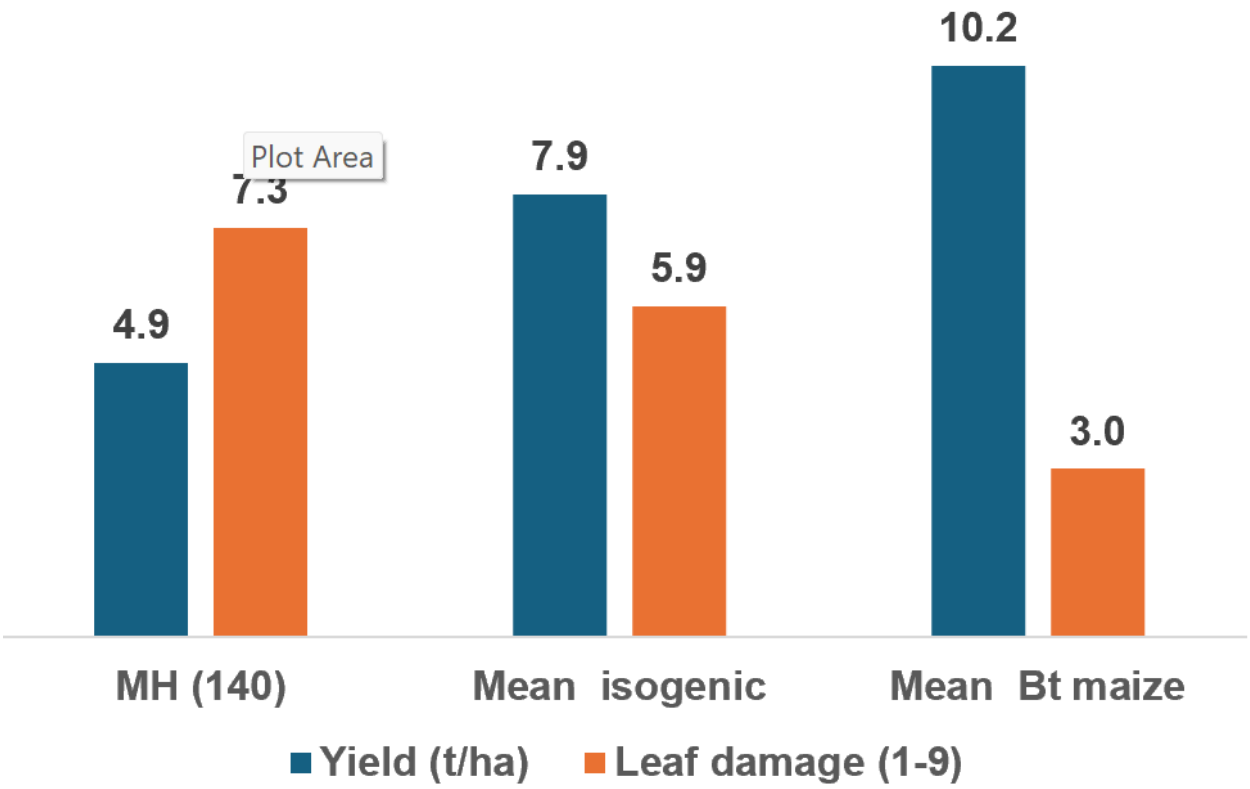


Figure 3: Mean performance Bt MON89034 hybrids under natural insect-pests infestation, at Melkassa, Ethiopia (2024)

Table 3. Mean FAW leaf damage score and other yield related components of Bt maize over the non-Bt isolines and best check under natural infestation of FAW and stem borer at Melkassa CFT site in 2024

Genotype	GY	DA	DS	PH	LD	EA	GYAI (%)	GYAC (%)
Bt_01	9.3	61.9	61.7	189.7	1.4	3.7	86.0	89.8
Isogenic_01	5.0	63.0	64.2	193.8	7.5	2.5		
Bt_02	7.2	61.5	65.8	181.4	1.8	2.2	63.6	46.9
Isogenic _02	4.4	62.5	66.6	179.9	7.2	2.2		
Bt_03	11.1	66.1	63.8	213.0	4.9	1.8	15.6	126.5
Isogenic _03	9.6	65.9	67.1	215.5	6.2	2.8		
Bt_04	9.6	64.5	62.9	206.3	3.1	3.7	7.9	95.9
Isogenic _04	8.9	64.4	62.2	194.2	7.4	3.4		
Bt_05	11.6	66.1	61.9	204.7	3.3	2.9	24.7	136.7
Isogenic _05	9.3	65.0	65.9	197.1	7.0	3.4		
Bt_06	12.6	67.0	65.9	208.8	3.6	3.4	22.3	157.1
Isogenic _06	10.3	68.0	64.3	201.4	7.4	3.1		
MH140	**4.9**	**66.7**	**64.5**	**206.2**	**7.3**	**2.1**		
Mean Bt	**10.2**	**64.5**	**63.7**	**200.7**	**3.0**	**3.0**	**29.1**	**108.1**
Mean Non Bt	**7.9**	**64.8**	**65.1**	**197.0**	**5.9**	**2.9**		
Heritability	1.0	0.9	0.9	0.9	1.0	0.9		
Genotype Variance	6.7	4.3	3.6	153.5	5.3	0.5		
Residual Variance	2.1	2.3	3.3	76	0.8	0.3		
Grand Mean	8.8	64.8	64.3	198	5.4	2.9		
LSD	1.6	1.7	2.0	9.7	1.0	0.6		
CV	16.3	2.3	2.8	4.4	16.7	18.9		
n Replicates	6	6	6	6	6.0	6.0		
Genotype significance	<00.1	<00.1	<00.1	<00.1	<00.1	<00.1		
P(0.05)	**	**	**	**	**	**		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm, EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

Table 4. Mean grain yield, leaf damage by FAW and other yield related components of Bt maize over the non-Bt isolines and best check under optimum condition at Melkassa CFT site in 2024.

Genotpe	GY	DA	DS	PH	EA	LD	EA	GYAI (%)	GYAC (%)
Bt_01	6.5	70	70	192.5	3.2	1.5	3.2	-11.0	-5.8
Isogenic_01	7.3	63	63	201.7	2.3	1.3	2.3		
Bt_02	5.6	65	64	194.5	2.0	1.1	2.0	-14.3	-18.8
Isogenic _02	6.4	64	65	194.0	2.5	1.1	2.5		
Bt_03	8.3	70	69	217.0	2.1	1.3	2.1	2.5	20.3
Isogenic _03	8.1	70	69	216.5	2.4	1.1	2.4		

Bt_04	8.4	65	64	214.5	3.1	1.1	3.1	6.3	21.7
Isogenic _04	7.9	66	64	214.5	2.3	1.5	2.3		
Bt_05	7.9	67	66	200.7	2.4	1.5	2.4	-3.7	14.5
Isogenic _05	8.2	66	65	199.6	3.1	1.1	3.1		
Bt_06	8.4	69	68	208.8	2.7	1.5	2.7	-3.4	21.7
Isogenic _06	8.7	71	70	207.8	2.9	1.7	2.9		
MH140	**6.9**	**71**	**69**	**210.9**	**2.5**	**1.1**	**2.5**		
Mean Bt	**7.5**	**67.9**	**67.0**	**204.7**	2.1	**1.4**	2.1	-3.8	8.7
Mean Non Bt	**7.8**	**66.8**	**66.0**	**205.7**	0.8	**1.3**	0.8		
Residual Variance	5.4	3.7	7.9	92.8	0.1	0.1	0.1		
Grand Mean	7.6	67.6	4.8	205.0	2.6	1.3	2.6		
LSD	2.7	3.0	66.8	14.2	0.6	0.5	0.6		
CV	30.7	2.8	3.4	4.7	14.4	28.7	14.4		
n Replicates	3.0	3.0	3.3	3.0	3.0	3.0	3.0		
Genotype significance	0.1	<0.01	3.0	<0.01	<0.01	<0.01	<0.01		
P(0.05)	ns	**	ns	**	**	**	**		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm, EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

b) Evaluation of efficacy of GM insect protection trait (Bt MON89034) on maize growth, grain yield, and pests' protection in Wondonet and Bako (Mid altitude high potential agro ecology)

The result showed that the Bt maize consistently displayed high relative grain yield and the lowest foliar damage at each location. At Wondogent (**Table 5**), the Bt maize hybrids (event MON89034) exhibited the highest relative grain yield advantage of 54.1% over the check and 9.6 % over its isogenic counterparts. The lowest mean leaf damage (1.7) also demonstrated by the Bt maize whereas the isogenic and check exhibited the highest mean leaf damage score of 5.7 and 6.4, respectively. The details are described in **Figure 4** and **Table 5**.

At Bako (**Table 7**), Bt maize hybrid (event MON89034) had relatively higher relative grain yield advantage of 8.5 % as compared to its isogenic hybrids and 17.4 %. However, the commercial check beats Bt hybrids with 20.3 % yield advantage regardless of some insect damage by FAW **(Figure 5)**. This indicates that the damage by FAW in Bako area was not severe and the current check is well adapted to the environment.

Regarding optimum-trial condition with pests controlled at each location, the Bt maize and their isogenic hybrids were almost similar (**Table 6** and **8**) and the result also confirmed that the

effectiveness of the Bt MON89034 gene had as good as chemicals in controlling the two distractive insect.

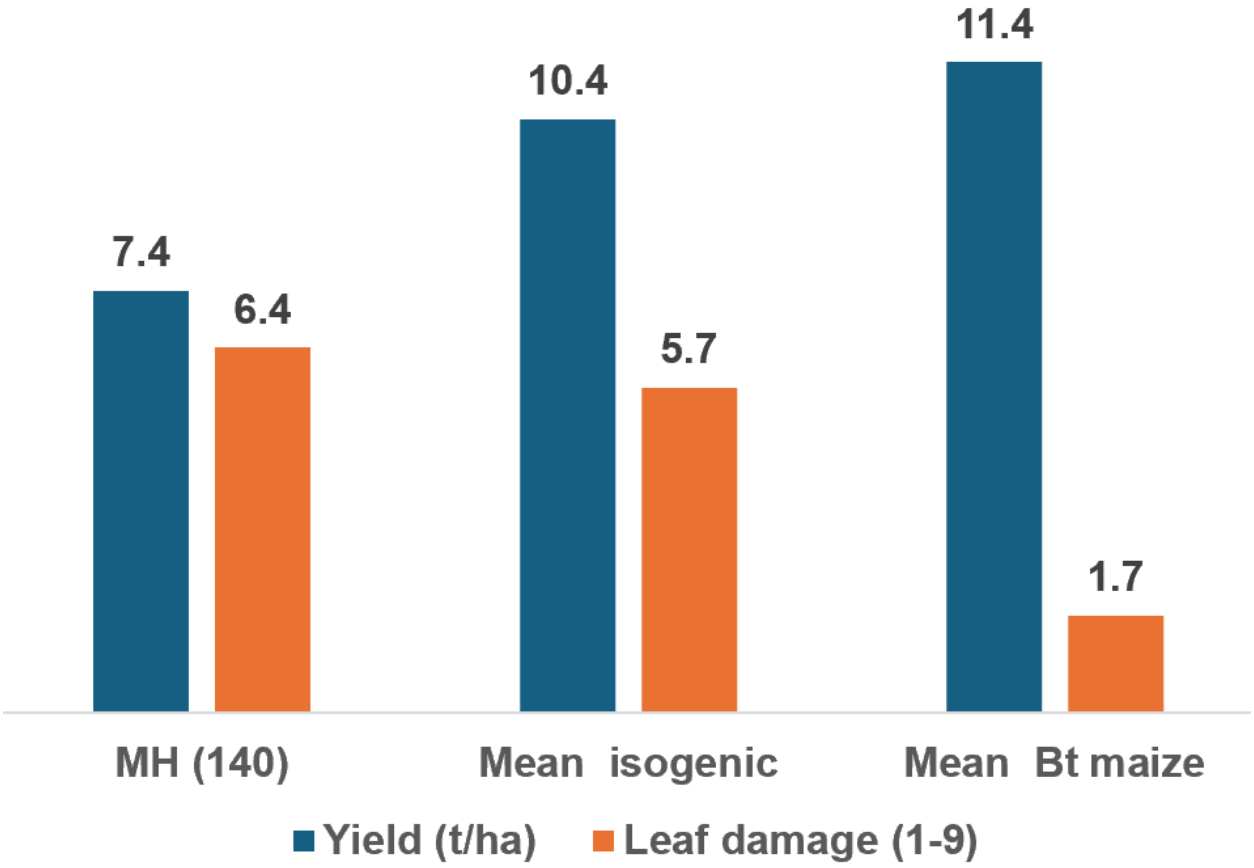


Figure 4: Mean performance Bt MON89034 hybrids under natural insect-pests infestation, at Wondogenet, Ethiopia (2023/24)

Table 5. Mean grain yield, leaf damage by FAW and other yield related components of Bt maize over the non-Bt isogenic hybrids and best check under natural infestation of FAW and stem borer at Wondogent CFT site in 2024.

Genotype	GY	DA	DS	PH	LD	EA	**GYAI (%)**	**GYAC (%)**
Bt_01	11.1	67.1	65.8	211.9	1.1	3.6	4.7	50.0
Isogenic_01	10.6	67.8	67.0	206.9	5.1	3.3		
Bt_02	8.8	66.3	65.5	192.8	1.1	3.9	0.0	18.9
Isogenic _02	8.8	65.6	65.0	198.9	6.5	2.9		
Bt_03	12	69.8	69.0	231.0	3.3	4.0	9.0	62.2
Isogenic _03	11	70.3	69.4	223.0	6.1	3.7		
Bt_04	12	70.3	69.4	223.0	1.1	3.4	10.1	62.2
Isogenic _04	10.9	69.4	68.5	219.0	5.3	3.4		
Bt_05	11.9	71.2	70.2	213.9	1.2	2.6	22.7	60.8
Isogenic _05	9.7	72.6	71.7	210.9	6.4	2.8		
Bt_06	12.7	72.4	71.4	219.0	2.1	2.4	10.4	71.6
Isogenic _06	11.5	72.7	71.7	221.0	4.5	2.5		

MH140	7.4	**70.1**	**68.8**	**222.0**	**6.4**	**3.0**		
Mean bt maize	11.4	**69.7**	**68.7**	**212.9**	**1.7**	**3.2**	**9.6**	**54.1**
Mean isogenic	10.4	**69.7**	**68.8**	**212.8**	**5.7**	**2.7**		
Heritability		0.9	0.9	0.6	1.0	0.9		
Genotype Variance		5.7	5.5	171.5	5.4	0.5		
Residual Variance		3.3	3.2	677.1	1.6	0.5		
Grand Mean		69.8	68.8	214.9	4.0	3.1		
LSD		2.0	2.0	23.3	1.4	0.8		
CV		2.6	2.6	12.1	31.4	23.3		
n Replicates		6.0	6.0	6.0	6.0	6.0		
Genotype significance		<0.01	<0.01	<0.01	<0.01	<0.01		
P (0.05)		***	***	***	***	***		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm, EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

Table 6. Mean grain yield, leaf damage by FAW and other yield related components of Bt maize over the non-Bt isogenic and best check under optimum condition at Wondogent CFT site in 2024.

Genotype	**GY**	**DA**	**DS**	**PH**	**LD**	**EA**	**GYAI (%)**	**GYAC (%)**
Bt_01	11.5	69.3	68.2	215.7	1.1	3.4	0.9	-3.4
Isogenic_01	11.4	75.1	73.9	215.7	1.9	3.4		
Bt_02	11.4	72.1	70.8	215.7	1.1	3.3	-0.9	-4.2
Isogenic _02	11.5	71.5	70.2	215.7	1.5	3.3		
Bt_03	11.4	72.4	71.1	215.7	1.8	3.4	1.7	-4.2
Isogenic _03	11.2	72.6	71.7	215.7	1.9	3.3		
Bt_04	11.9	69.1	67.9	215.7	1.6	3.4	10.2	0.0
Isogenic _04	10.8	65.5	64.2	215.7	1.1	3.4		
Bt_05	11.5	66.3	65.1	215.7	1.9	3.4	6.5	-3.4
Isogenic _05	10.8	69.5	68.9	215.7	1.4	3.4		
Bt_06	11.8	71.3	69.7	215.7	1.7	4.1	5.4	0.8
Isogenic _06	11.2	72.1	70.2	215.7	1.1	3.4		
MH140	**11.9**	**72.4**	**71.1**	**215.7**	**1.1**	**3.4**		
Mean Bt maize	**11.6**	**70.1**	**68.9**	**215.7**	**1.1**	**3.4**	**3.6**	**-2.5**
Mean Isogenic	**11.2**	**71.0**	**69.8**	**215.7**	**1.8**	**3.5**		
Heritability	0.9	0.8	0.9	0.0	0.8	0.1		
Genotype Variance	1.4	8.0	7.5	0.0	0.2	0.6		
Residual Variance	0.5	5.0	3.8	1303.5	0.1	24.6		
Grand Mean	9.0	70.9	69.6	215.7	1.5	3.4		
LSD	1.2	3.6	3.2	0.0	0.5	2.2		
CV	7.7	3.2	2.8	16.7	21.0	144.9		
n Replicates	3.0	3.0	3.0	3.0	3.0	3.0		
Genotype significance	<0.01	<0.01	<0.01	1.0	<0.01	0.9		
P(0.05)	***	***	***	Ns	***	ns		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm, EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

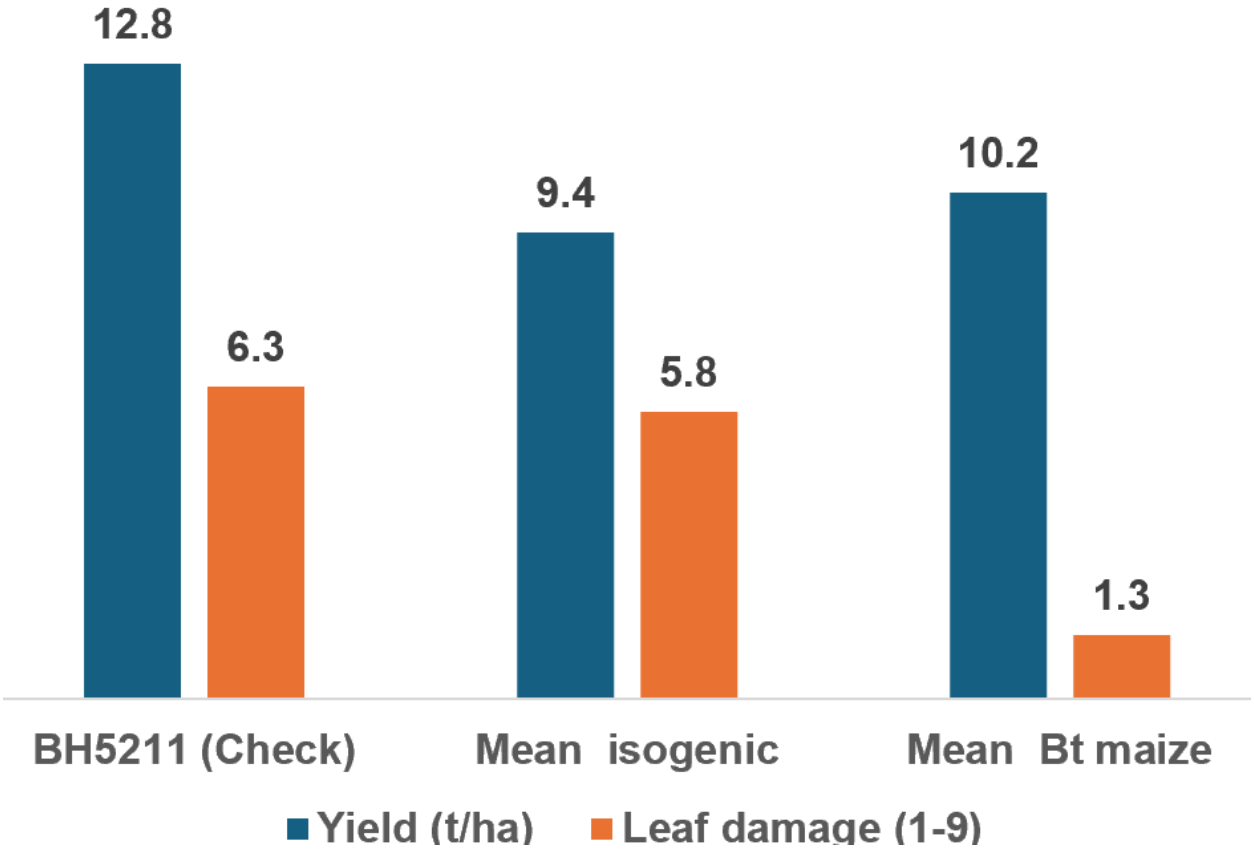


Figure 5: Mean performance Bt MON89034 hybrids under natural insect-pests infestation, at Bako, Ethiopia (2024)

Table 7. Mean grain yield, leaf damage of by FAW and other yield related components of Bt maize over the non-Bt isogenic hybrids and best check under natural infestation of FAW and stem borer at Bako CFT site in 2024

Genotype	**GY**	**DA**	**DS**	**PH**	**LD**	**EA**	**GYAI (%)**	**GYAC (%)**
Bt_01	9.6	71.4	71.4	234.5	1.1	6.5	2.1	-25
Isogenic_01	9.4	71.4	71.7	234.5	5.6	6.6		
Bt_02	6.8	70.6	70.9	216.5	1.1	6.6	13.3	-46.9
Isogenic _02	6.0	71.1	71.3	208.4	5.8	6.8		
Bt_03	10.0	71.6	72.1	243.1	2.2	5.6	27.9	-21.9
Isogenic _03	8.6	73.3	73.2	240.0	4.8	6.1		
Bt_04	9.2	74.3	74.8	240.3	1.1	5.2	5.7	-28.1
Isogenic _04	8.7	74.1	75.1	237.2	5.6	5.5		
Bt_05	11.8	73.7	74.3	236.7	1.1	4.2	1.7	-7.8
Isogenic _05	11.6	72.7	73.2	230.5	6.4	4.3		
Bt_06	14.0	73.2	73.8	251.1	1.1	2.2	16.7	9.4
Isogenic _06	12.0	72.7	73.8	241.7	6.6	2.9		
BH5211	**12.8**	**75.9**	**76.4**	**280.3**	**6.3**	**2.7**		
Mean Bt maize	**10.2**	**72.9**	**73.5**	**240.1**	**4.5**	**4.8**	**8.5**	**-20.3**
Mean Isogenic	**9.4**	**73.9**	**74.2**	**241.9**	**3.5**	**4.5**		
Heritability		1.0	1.0	1.0	1.0	1.0		
Genotype Variance		5.7	5.6	398.2	6.1	3.1		
Residual Variance		1.1	1.2	45.0	1.3	0.4		
Grand Mean		73.2	73.7	241.1	3.9	4.8		
LSD		1.2	1.3	7.7	1.3	0.8		
CV		1.5	1.5	2.8	28.9	13.7		
Genotype significance		<0.01	<0.01	<0.01	<0.01	<0.01		
P(0.05)		***	***	***	***	***		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm, EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

Table 8. Mean grain yield, leaf damage by FAW and others yield related components of Bt maize over the non-Bt and best check under optimum condition at Bako CFT site in 2024.

Genotype	GY	DA	DS	PH	LD	EA	**GYAI (%)**	**GYAC (%)**
Bt_01	9.7	71.6	71.6	244.6	5.0	5.0	5.4	-26.0
Isogenic_01	9.2	71.6	71.6	244.9	5.0	5.0		
Bt_02	7.2	72.5	72.2	230.9	7.3	6.6	-6.5	-45.1
Isogenic _02	7.7	72.8	73.1	223.9	7.3	6.3		
Bt_03	10.0	72.5	72.5	257.8	6.0	5.3	-11.5	-23.7
Isogenic _03	11.3	72.2	72.8	263.9	6.0	5.0		
Bt_04	8.8	74.6	75.9	242.9	5.0	5.6	-4.3	-32.8
Isogenic _04	9.2	74.6	75.9	248.7	5.3	5.3		
Bt_05	11.9	74.3	75.6	235.3	3.4	4.0	8.1	-9.2

Isogenic _05	11.0	74.0	75.3	242.0	3.4	4.3		
Bt_06	13.6	74.6	75.6	258.1	2.4	2.4	-6.8	3.8
Isogenic _06	14.6	74.3	74.7	253.7	2.4	2.4		
BH5211	**13.1**	**78.0**	**78.6**	**288.5**	**1.4**	**2.4**		
Mean Bt maize	**10.8**	**74.2**	**74.7**	**251.4**	**4.1**	**4.3**	**-3.6**	**-17.6**
Mean Isogenic	**11.2**	**74.5**	**75.2**	**248.7**	**4.1**	**4.2**		
Heritability	0.9	0.9	0.9	0.9	1.0	1.0		
Genotype Variance	7.5	5.5	6.7	402.3	4.0	2.4		
Residual Variance	1.7	1.6	1.9	168.9	0.3	0.3		
Grand Mean	10.9	74.0	74.6	251.6	4.4	4.4		
LSD	2.1	2.0	2.2	20.4	1.0	0.8		
CV	12.0	1.7	1.8	5.2	13.3	11.4		
Genotype significance	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
P(0.05)	***	***	***	***	***	***		

GY=Grain Yield (t/ha), DA=Male Flowering (day), DS=Female Flowering (day), PH= Plant Height (cm), EA= Ear Aspect (1-9 Scale), LD= Leaf Damage by FAW (1-9 Scale), GYAI= Relative Grain Yield Advantage over Isogenic Hybrid (%), GYAC= Relative Grain Yield Advantage over Check (%)

vii) Results of confined field trials from other TELA Project countries

In Nigeria and the Republic of South Africa the a similar results were found and maize stacked with MON89034 events showed superior performance in grain yield advantage and less leaf damage against their counter part of isohybrids and commercial checks under the target pest infestation.

In Nigeria, the results of CFTs demonstrated that MON89034 Bt maize hybrids displayed 19% higher yield than their non-GM isogenic versions, and 40% higher yield than the commercial checks. In sharp contrast, the Bt maize hybrids recorded ≤ 2 leaf damage relative to their non-GM isogenic versions which scored ≥ 4 and the study conferring resistance of MON89034 events against stem borer and fall armyworm. (Muhyideen Oyekunle *et. al*, 2023).

In south Africa, MON 89034 maize, was commercialized in 2011. MON 89034 had increased to 75%, in the 2021–2022 season. Various assessments of the susceptibility target pest to MON 89034 Bt maize carrying Cry1A.105 and Cry2Ab2 were done between 2008 (prior to regulatory approval and commercial deployment) and 2016 in South Africa, The result demonstrated that no larval survival was reported. MON 89034 maize therefore provided effective protection against the target pest (Elrine Strydom *et. al*., 2024)

Conclusions

In general, releasing and commercialization of insect-protected maize stacked with MON 89034 event will ensure sustainability of maize production and productivity in Ethiopia. Moreover, the Bt maize technology helps to reduced chemical pesticide usage; pesticides impact on human health and the environment; reduced mycotoxin level in corn kernels; and no additional labor or machinery required thereby allowing both large and small growers to maximize hybrid yields. Hence, it is reasonable to have environmental release to verify and commercialize MON89034 stacked Bt maize in Ethiopia.

10(3). Trait transfer capability:

i) Possibility for post-release transfer of inserted genetic materials from the modified organism into organisms in the ecosystem;

Maize with event MON 89034, like all other maize, is not sexually compatible with any indigenous or introduced wild plant species present in Ethiopia. Therefore, the potential for genetic transfer and exchange with other plants is limited to cross-pollination to cultivated maize plants. All maize can inter-pollinate. Section 7(2)(n) above provides a detailed risk assessment related to potential gene flow.

The risk assessment leads to a conclusion that, there is no potential for gene transfer from maize with event MON 89034 to wild plant species since there are no wild types of maize reported in the country, Ethiopia and thus the likelihood of transferring genes from such wild types sources is negligible. In the event that the introduced genes would outcross to another maize plant, its transfer would, in any event, have negligible consequences for the environment. The environmental risk posed by this transfer, and hence by the intended cultivation and use of the genetically modified maize for grain food, feed and processing is negligible.

ii) Possibility for post-release transfer of genetic material from organisms in the ecosystem to the released modified organism;

As stated above, maize with event MON 89034, like all other maize, is not sexually compatible with any indigenous or introduced wild plant species present in Ethiopia. Therefore, the potential for genetic transfer and exchange with other plants is limited to cross-pollination to cultivated maize plants. All maize can inter-pollinate. Maize pollen of a specific hybrid cultivar can be carried by the wind and could fertilize other cultivars. In the event where the modified maize germinates, matures, produces pollen and fertilizes a neighboring crop, the traits present in maize with event MON 89034 could be transferred to the recipient maize crop, including the introduced trait, and would be expressed in the progeny of the recipient crop.

The possibility of post-release transfer of genetic material from organisms in the ecosystem to the released genetically modified (GM) organism MON 89034 (a Bt maize variety expressing insecticidal Cry proteins) depends on several biological and ecological factors. In nature, the transfer of genetic material from wild plants or microbes to MON 89034 is highly unlikely due to biological barriers. Plants do not naturally exchange genetic material horizontally (unlike bacteria). MON 89034 could receive genetic material from other maize plants (including other GM maize) via cross-pollination. However, this is vertical (sexual reproduction), not horizontal transfer. Soil and plant-associated microbes could take up transgenic DNA, but stable integration and functional expression are rare and environmentally insignificant. EFSA & EPA assessments have concluded that horizontal gene transfer (HGT) from GM plants like MON 89034 to other organisms is negligible in the environment. The likelihood of functional genetic material transferring from wild organisms to MON 89034 post-release is negligible under natural conditions. While hypothetical scenarios (e.g., bacterial transformation with transgenic DNA) exist, they lack ecological relevance due to low frequencies and lack of selective advantage (EFSA, 2019; EFSA, 2010; EFSA, 2008).

10(4). Likelihood of post-release interaction leading to the expression of unexpected or undesirable traits in the modified organism or in its products;

The potential for genetic transfer and exchange with other plants is limited to cross-pollination to cultivated maize plants. The outcrossing potential has been discussed above in sections 7(2)(n) and 10(3)a(iii). It is noted that the potential for transfer of genetic material between maize crops is limited by the mobility of maize pollen.

In the case where a trait would be transferred, the risk of the drought-tolerance and insect pest protection traits to be the cause of any meaningful competitive advantage or disadvantage that could impact the receiving environment is negligible.

10(5). Measures employed to ensure and verify genetic stability; description of genetic traits which may prevent the transfer of genetic materials; methods used to verify stability;

As elaborated in section 2(c) above, the inserted genes in the modified maize are stably integrated into maize genome. This was demonstrated by the fact that the Southern blot assays of the modified maize and indicated that stability was maintained for several generations. The stability was further confirmed by the fact that the inheritance of the gene insert follows Mendelian patterns of segregation. Segregation analyses show heritability and stability of the transformation genes occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

10(6). Routes of biological dispersal of the modified organism, known or potential modes of interaction with the disseminating agents, including dispersal by wind, water, inhalation, ingestion, surface contact with or burrowing into animals, and others;

Maize is an annual crop and seeds are the only survival structures and means of propagation. Natural regeneration from vegetative tissue is not known to occur. Maize has a polystichous female inflorescence (ear) on a stiff central spike (cob) enclosed in husks (modified leaves), which limit the dispersal of seed from the cob. Consequently, natural dispersal of individual grains does not occur. Maize has no biological mechanism to scatter seed, but low-level, incidental dispersal can occur as a result of human-based activities such as transport and harvesting operations. As was noted by the OECD (2003), the few plants that might result from incidental release will not persist or meaningfully reproduce without human intervention.

10(7). Description of ecosystem to which the modified organism could be disseminated

As stated in section 8.3, maize with event MON 89034 has been developed for cultivation in all maize growing regions of Ethiopia to address challenges brought about by drought stress and stem borer pests, including fall armyworm.

11. Potential environmental impacts

11(1). Potential for excessive population increase of the modified organism in the environment;

As mentioned above, maize is propagated through seed and generally is a weak competitor. In non-agricultural environments, the selective advantage presents negligible risk because of the poor

survival characteristics of maize outside cultivation. Indeed, in the unlikely case where kernels would germinate outside of the field, the traits for drought tolerance and insect pest protection would not confer any meaningful advantage to the emerging plant, as maize is incapable of surviving without human assistance in the wild.

11(2). Competitive advantage of the modified organism in relation to the unmodified or parental species;

The modified maize with event MON 89034is equivalent to the unmodified maize except for the additional traits for insect pest protection.

11(3). Identification and description of the target species that are anticipated to be affected by the modified organism;

Event MON 89034 codes for production of an insecticidal proteins, Cry1A.105 and the Cry2Ab2 , that offers protection against feeding damage caused by lepidopteran insect pests such as stem borer species *Busseola fusca*, and *Chilo partellus*, and fall armyworm (FAW), Spodoptera frugiperda.

11(4). Anticipated mechanism and result of interaction between the released modified organism and the target species;

As explained above, maize with event MON 89034 expreses Cry1A.105 and the Cry2Ab2 proteins which confers protection against crop damage by certain lepidopteran insect pests. The general mechanism of insecticidal activity of the Cry proteins is well understood. A generalized mode of action of Cry1Ab protein includes the following steps: i) ingestion of the protoxin crystal by the insect; ii) solubilization of the crystal in the insect midgut; iii) proteolytic processing of thetarget released Cry protein by digestive enzymes to produce an active toxin termed delta-endotoxin; iv) binding of the endotoxin to receptors on the surface of midgut epithelial cells of target organisms; v) formation of membrane ion channels or pores and vi) and consequent disruption of cellular homeostasis (English, 1992). Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die (Sacchi et al., 1986).

11(5). Identification and description of non-target species which may be affected;

Maize with event MON 89034 expresses the introduced Cry1A.105 and the Cry2Ab2 protein, protecting the plants against herbivorous predation by important insect pests of maize. It has been established that this Cry protein exhibits selective toxicity towards certain lepidopteran pests, but not against other insect orders. This specificity is consistent with findings reported in the published literature, that demonstrate that proteins of the Cry1A.105 and the Cry2Ab2 class exhibit lepidopteran selective toxicity (Aronson and Shai, 2001; Crickmore et al., 1998; Crickmore et al., 2005; Dulmage, 1981; Klausner, 1984; MacIntosh et al., 1990; Mendelsohn et al., 2003; Romeis et al., 2006; Whiteley and Schnepf, 1986).

11(6). Likelihood of post-release shifts in biological or in target range;

Stem borers and FAW are associated with graminae and the targets in this application attack mainly maize. Cry1Ab that confers insect protection has some effect of other Lepidopteran pests and there are some reports indicating that it could reduce populations of the fall armyworm, which is currently

a contraint in maize fields in Ethiopia and other parts of Africa. Any shift to control some other pests would be an advantage to farmers and is not unexpected. Besides, no such unfavorable post-release shifts in biological or in target range has been observed in the 24 years that Bt maize has been grown since first release in 1996 (James 2018).

11(7). Known or predicted effects on population levels of competitors, preys, hosts, symbionts, predators, parasites and pathogens;

Effect of the transformed maize on biodiversity including; competitors, preys, hosts, symbionts, predators, parasites and pathogens has been studied.

Primary consumers typically are macro-organisms that feed on the detritus, i.e. the organic debris resulting from decomposition of plant material. They further reduce the size of the detritus particles through partial digestion, and after defecation of the particles, thereby enhancing further decomposition. Detritivores also provide aeration to the soil or litter material, thereby improving the oxygen content in the soil and increasing respiration of decomposers. Important examples of detritivores are springtails (Collembola), millipedes and annelid worms. Typically, populations of soil-borne consumers are significantly affected by plant genotype, tillage practices, environmental conditions, previous history of crops grown and the application of pesticides and fertilisers (Alexander, 1961; Tiedje et al., 1999; Tabashnik, 2008). The Cry1A.105 and the Cry2Ab2 protein expressed in maize with events MON89034 was demonstrated to have negligible risk or adverse environmental effects through direct or indirect interactions with non-target organisms, including representative detritivores that are involved in the decomposition function in the soil (US EPA, 2001).

Decomposers include bacteria and fungi (saprophytes) that break down dead and decaying material, such as stubble plant material, fresh litter remaining after harvest of the crop, smaller detritus from more advanced decomposition and humus. Dead and decaying plant material contains important nutrients, e.g. carbon which is released as carbon dioxide as a result of microbial respiration, and nitrogen which is recycled by a range of soil bacteria. Bacterial and fungal populations are critical to maintaining soil health and quality.

Soil microbial communities that mediate biogeochemical processes are highly complex and are often characterized by high microbial diversity (Tiedje et al., 1999). However, the diversity and abundance of these organisms and hence their microbial processes are significantly affected by biotic factors (community characteristics and dynamics), abiotic factors (soil structure, clay type, moisture capacity, environmental conditions, pH) and soil use (crop type, tillage practices and history of previously grown crops). Agricultural practices such as fertilization and cultivation techniques may also have profound effects on soil microbial populations, species composition, colonization and associated biochemical processes (Alexander, 1961). Consequently, significant variation in microbial populations is expected in the agricultural environment.

The toxic mechanism of Cry1A.105 and the Cry2Ab2 proteins are well characterised and was found to be extremely specific to larvae of certain lepidopteran insect pests. Consequently, the potential for activity of this protein towards microorganisms is negligible. In addition, as expected, the Cry1Ab protein was shown to degrade rapidly in soil, which confirms the absence of adverse effects on soil microorganisms. The degradation rate of the Cry1Ab protein was assessed by measuring the decrease

in insecticidal activity of MON 89034 tissue incubated in soil. This rapid degradation strongly supports the lack of exposure of Cry1A.105 and the Cry2Ab2 on non-target organisms involved in the decomposition function and on soil-dwelling organisms in general (Zhang et al., 2015). Finally, extensive experience with the commercialization of various Cry1A.105 and the Cry2Ab2-expressing insect-protected crops has not revealed any adverse effects.

In conclusion, as for conventional maize, it is highly unlikely that there would be any significant immediate or delayed adverse effects from maize with event MON 89034.

11(8). Known or predicted involvement of the modified organism in bio-geochemical processes;

As elaborated above, maize production in general is known to have indirect impacts on biogeochemical processes through tillage, fertilizer application and establishment of a monoculture (where grown as sole crop) in a defined area. Since maize with event MON 89034 was shown to be compositionally equivalent to conventional maize and was not different in morphology, development, yield, dissemination, stress susceptibility, plant health and survival characteristics, there is no evidence that it would be any different from conventional maize regarding its direct influence on nutrient levels in the soil. Expression of the newly introduced Cry1A.105 and the Cry2Ab2 proteins is a characteristic of genetically modified maize that could, potentially, cause an adverse environmental effect on biogeochemical processes.
If the introduced proteins exerted a potential to cause adverse effects on populations of decomposers and/or detritivores in the soil, then the biogeochemical processes which these organisms are involved in could be affected, which could lead to a change of the nutrient cycles in the environment.

11(9). Other potentially significant interactions with the environment

Data on ecological interactions indicate that maize with event MON 89034 does not confer any increased susceptibility or tolerance to abiotic stressors that would be associated with increased ecological risk compared to conventional maize (see risk assessment document).

12. Information on monitoring and monitoring techniques

12(1). Methods for tracing the modified organism and for monitoring its effects;

Agronomic studies on maize with event MON 89034 have demonstrated that the introduced traits have not changed any of the typical characteristics of maize. Survival of seed from MON 89034 maize would therefore be the same as that of seed from any conventional maize.

While a range of insect control products exist, over time insects evolve to develop coping mechanisms, which contribute to development of resistance against the products used. A successful pest management program would necessary be backed by routine inspection and monitoring for efficacy. Thus, maize with event MON 89034 will be commercialized alongside quality control and monitoring measures encompassing an IRM plan and a stewardship program in order to secure the valuable agronomic and other benefits of insect-protected maize on a longer term. The applicant will institute a monitoring program in line with the requirements of the relevant Directives.

To trace and monitor MON 89034, a combination of molecular, protein-based, phenotypic, and documentary methods is used. Event-specific PCR and quantitative real-time PCR (qPCR) detect the

unique DNA insertion to confirm its presence, while ELISA and lateral flow strips identify Cry1A.105 and Cry2Ab2 proteins for rapid field testing. Phenotypic monitoring includes insect bioassays to verify toxin functionality and herbicide tolerance checks if stacked with other traits.

The IRM and Stewardship plans are briefly described below:

Insect resistance management plan for deployment of maize with event MON 89034 in Ethiopia

The evolution of resistance to the Bt toxins by insect pests is a major concern in application of Bt technology. However, the rate of evolution of this resistance can be slowed or stopped through the use of appropriate and integrated IRM strategies. Results from both laboratory and confined field trials indicate that the cry1Ab gene in MON89034 hybrids had a positive and significant effect on yield and significantly reduced the larvae survival of stem borer and FAW insects, *Sesamia calamistis*, *Busseola fusca* and *Chilo partellus*. However, to reduce the possibility of resistance development to MON 89034 hybrids, an IRM plan was developed. The IRM plan conforms to the existing cropping systems, and the components are intended to be adaptable and socially acceptable to the target farmers.

The elements of this IRM plan include: i) structured refuge of non-Bt maize variety in an adequate level in the vicinity of MON 89034 hybrids to support a sufficient non-pressured population of susceptible target insects species; ii) monitoring of insect resistance on or near a Bt crop for early detection of field evolved resistance; iii) remedial action plan in case of any confirmed development of resistance; iv) sound integrated pest management (IPM) practices and alternative control measures and v) IRM plan implementation through farmer education and compliance monitoring programs for greater awareness of Bt maize cultivation and proper stewardship, respectively. If appropriately implemented by farmers through education and compliance monitoring programs, this IRM strategy will delay the development of resistance, thereby extending the efficacy of events MON 89034 in maize hybrids in Ethiopia.

Stewardship program for deployment of MON 89034 in Ethiopia

The WEMA/TELA maize project has developed a robust stewardship program that adopts a full life-cycle approach that addresses all major aspects of responsible product management. The aspects fall into three categories, namely, i) Regulatory compliance; ii) Product deployment and iii) Commercialization of seed. Key elements of these phases include, but not limited to, compliance, incidence response process, seed production, packaging, labelling, marketing, branding, sales, distribution, seed company training, farmer education, seed quality standards, communications, complaint handling process and product recall process, amongst others. As an example, if this product does not meet high quality standards post commercial release, there is a mechanism in place to take an appropriate action as soon as the complaint is received to investigate, trace the product through the supply chain and initiate a recall or withdrawal process in a timely and effective manner.

12(2). Specificity, sensitivity and reliability of the monitoring techniques to identify the modified organism;

As mentioned above in section 8, Part 1, Cry1Ab proteins in maize with event MON 89034 is detectable through several molecular techniques. Southern blot or PCR techniques can identify the inserted nucleotide sequences, while specific ELISAs have been developed to detect the presence of

expressed proteins in plant tissues. Event-specific PCR-assays allowing the detection and the quantification of gene inserts in maize with events MON 89034 have been developed and validated by the European Commission Joint Research Centre (EU JRC), and are available on the EU JRC website at: http://gmo-crl.jrc.ec.europa.eu/summaries. Techniques for detecting the transfer of the introduced transgene to other organisms;

12(3). Techniques for detecting the transfer of the introduced transgene to other organisms;

Details of the available Techniques for detections have been elaborated in sections 5, 7(2) and 8, of this application.

12(4). Methods for detecting aberrant expression of traits and their causes.

Methods for detection of aberrant expression of traits and their causes will be similar to those described above in section 12(2).

PART FOUR
ADDITIONAL INFORMATION REQUIRED FOR PLACING ON A MARKET A COMMODITY CONSISTING OF A MODIFIED ORGANISM

13. Information required where the transaction relates to an application for placing a modified organism on a market

13(1). Name of the commodity, the scientific and common names and the transformation event of the modified organism;

Common	Maize or Corn
Scientific	*Zea mays* L.
Commercial	TELA maize
Transformation event Code	MON 89034
Identification Code registered with the Information Exchange Mechanism	Unique identifier is MON-89034
Electronic address of the Information Exchange Mechanism	https://bch.cbd.int

13(2). Names and addresses, including telephone numbers and e-mails, of the maker and the distributor of the modified organism;

Name of Proponent (s) Address

Ethiopian Institute of Agricultural Research (EIAR) and African Agricultural Technology Foundation – AATF

1. EIAR

Address: P.O. Box: 31, West Showa Zone, Oromia Region	
Telephone:	(+251) 112370376
Email:	tesfayedisasa@yahoo.com
Telephone: Technical contact person	Tesfaye Disasa
Email: Technical contact person	Email:tesfayedisasa@yahoo.com

AATF

Address: Executive Director, African Agricultural Technology Foundation, ILRI Campus, Nairobi

Telephone: P. O. Box 30709-00100, Nairobi +254 (020) 4223745/25	**Fax**: +254 204223701
Email :	c.kanangire@aatf-africa.org
Technical contact person **Telephone**: Tel: +254 735 992203: +254 204223700 **Email**: f.nangayo@aatf-africa.org	Dr. Francis Nang'ayo): Web: www.aatf-africa.org

Collaborating Institutions: Roles and responsibilities

Organization	Expertise	Roles and Responsibilities
AATF	Private-public partnership management, Project leadership and coordination	Facilitate negotiations of all agreements to access proprietary and allied technologies
		Coordination of Project activities across partner institutions
CIMMYT	Conventional, abiotic and biotic stress breeding, molecular and doubled haploid breeding	Provide leadership in maize breeding
Bayer Crop Science	Conventional, molecular and doubled haploid breeding	Provide global germplasms for use in maize breeding, Provide leadership in maize DNA marker information.
	Biotechnology testing and stewardship, Seed production, deployment and licensing	Donate transgenic traits for drought and insect protection to WEMA/TELA Project, Support formulation and implementation of seed deployment strategy
EIAR	Field testing for breeding and regional trials	Provide locally adapted germplasms to breeding component, Participate in maize breeding, Conduct confined field trials for testing transgenic WEMA products.
	Knowledge of farmers' product needs	

13(3). Specification for accessing information on the modified organism in the Biosafety Clearing House of the Protocol;

Common	Maize or Corn
Scientific	*Zea mays* L.
Commercial	TELA maize
Transformation event Code	MON 89034
Identification Code registered with the Information Exchange Mechanism	Unique identifier is MON-89034-4
Electronic address of the Information Exchange Mechanism	https://bch.cbd.int

13(4). Type of expected use;

Maize with event MON 89034 will be used for purposes similar to those of conventional maize including consumption as direct food, feed, processing and for export.

13(5). Specific instructions for storage and handling.

There are no unique storage and handling requirements. All labelling and handling will follow guidelines provided by the regulator.

A common storage facility can be used for MON 89034 maize as long as proper labeling and segregation practices are followed to prevent commingling with non-GM or other GM varieties. Hence, MON 89034 does not require unique storage conditions. However, clear labeling (e.g., "Genetically Modified MON 89034" with a unique identifier number must be displayed on storage bins, bags, and transport documents to ensure traceability. Proper documentation, including lot numbers and GMO declarations, ensures compliance with international trade requirements even when using shared storage facilities.

14. Information required where the transaction relates to the placing of modified organism as food on the market

14(1). Equivalence studies;

Compositional analyses of forage and grain tissues from maize event MON 89034, comparing key nutrients, anti-nutrients, and secondary metabolites to conventional maize, confirmed that maize with event MON 89034 is compositionally and nutritionally equivalent to conventional maize hybrids. The analysis, conducted in 2004 at five U.S. field sites, included 15 conventional corn hybrids as references to establish a 99% tolerance interval for each component analyzed, accounting for natural variability in commercial corn. This approach ensures that any statistically significant differences between MON 89034 and the conventional control can be assessed in terms of biological relevance, confirming the safety and equivalence of MON 89034 to current maize varieties (Drury et al., 2008).

14(2). Information on antibiotic resistance marker genes (if used);

The NptII protein serves as a selectable marker during the early stages of plant cell selection after transformation (De Block et al., 1984; Horsch et al., 1984). It inactivates neomycin and related

antibiotics by phosphorylating them with ATP. This allows only cells producing the NptII enzyme to survive exposure to these antibiotics. The nptII gene, sourced from the E. coli transposon Tn5, is integrated into maize cells to enable effective selection following transformation (Beck et al., 1982). Since plant transformation efficiency is typically low, ranging from 1×10^{-5} to 1×10^{-4} of treated cells (Fraley et al., 1983), the NptII marker aids in the screening process.

14(3). Characterization of novel proteins or other novel substances which includes information about prior history of human consumption of the novel substances if any, or their similarity to substances previously consumed in food;

Cry1A.105 and Cry2Ab2 proteins, derived from *Bacillus thuringiensis*, have been evaluated for potential toxicity following Codex guidelines. These proteins are structurally and functionally similar to other Cry proteins with a safe history of use and do not share characteristics with known toxins or harmful proteins. Toxicity studies in mice showed no adverse effects, even at high doses, and these proteins constitute only a small fraction of the total protein in MON 89034 maize. Given maize's long history of safe use and the substantial equivalence of MON 89034 to conventional maize, no further testing of other constituents is required. Both cry protein, Cry1A.105 and Cry2Ab2, represent no more than 0.005% and 0.001% of the total protein in the grain of MON 89034, respectively. These findings suggest that Cry1A.105 and Cry2Ab2 proteins pose no significant risk to humans or animals (see, FSANZ, 2008).

14(4). The potential toxicity of novel proteins

Cry1A.105 is a chimeric protein derived from *Bacillus thuringiensis* (Bt), composed of three N-terminal domains (I, II, III) and a C-terminal region. Domains I and II are identical to Cry1Ab and Cry1Ac, while domain III is 99% identical to Cry1F, and the C-terminal region matches Cry1Ac. The overall amino acid sequence identity of Cry1A.105 is 93.6%, 90.0%, and 76.7% to Cry1Ac, Cry1Ab, and Cry1F, respectively. Cry2Ab2 is another Bt protein, and Bt strains producing Cry1 proteins have been used as biopesticides since 1997. Bt crops expressing these proteins, including Cry1Ac, Cry1Ab, Cry1F, and Cry2Ab2, have been widely cultivated without known reports of allergies or toxicity (see, Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

14(5). The potential Allergenicity of novel proteins

Cry1A.105 and Cry2Ab2 are proteins produced by *Bacillus thuringiensis* (Bt) and used in genetically modified crops for pest resistance. To assess their potential allergenicity, two approaches were employed. First, the source of the genes encoding these proteins, *Bacillus thuringiensis*, is not known to be allergenic, suggesting the proteins themselves are unlikely to be allergens. Second, the amino acid sequences of Cry1A.105 and Cry2Ab2 were compared to known allergens, and no significant similarity was found, further supporting the conclusion that these proteins are not likely to be allergens. Furthermore, Cry1A.105 and Cry2Ab2 proteins are quickly broken down in simulated gastric fluids, with over 95% to 99% being digested within 30 seconds. Proteins that are rapidly digested in the mammalian gastrointestinal system are unlikely to cause allergies when consumed (see, Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

14(6). Compositional analysis of the modified organism as food or food derived from a modified organism;

Compositional assessments of grain and forage from multiple U.S. corn production sites indicate that MON 89034 is nutritionally and compositionally equivalent to conventional corn. The analysis covered 77 components, including protein, fat, carbohydrates, vitamins, and minerals, with no significant differences found in 58 of them. While small statistical differences were noted in phosphorus (forage), stearic acid (grain), and arachidic acid (grain), these variations were minimal (3.4-19.2%) and fall within the natural variability ranges of commercial corn hybrids. Thus, the differences observed are not biologically meaningful, confirming that MON 89034 is compositionally equivalent to conventional corn (Drury et al., 2008).

14(7). Data to allow the nutritional impact of compositional changes in the food to be assessed;

MON 89034, genetically modified maize, introduces a trait of agronomic interest without altering its nutritional composition. Extensive compositional analyses confirmed its substantial equivalence to conventional maize, except for the introduced trait. A feed performance study using rapidly growing broiler chickens showed that diets containing MON 89034 grain did not affect the chickens' performance compared to control groups fed non-transgenic maize or commercial hybrids. The study confirms that MON 89034 is nutritionally equivalent to conventional maize and other maize varieties, with no unintended effects from the introduced trait. The compositional analysis of MON 89034 maize demonstrated nutritional equivalence to conventional maize, with proximates, fiber, minerals (e.g., manganese, phosphorus), amino acids, fatty acids (e.g., stearic acid), vitamins (e.g., B2), antinutrients (phytic acid, raffinose), and secondary metabolites (ferulic/p-coumaric acid) (See for detail, Drury et al., 2008).

14(8). Data from animal feeding study;

MON 89034 maize, which contains an introduced trait of agronomic interest, has been shown to be nutritionally equivalent to conventional maize. Extensive compositional analyses and a confirmatory feed performance study with rapidly growing broiler chickens demonstrated that MON 89034 does not differ from non-transgenic maize in terms of nutritional value or feed performance. The study also confirmed the absence of unintended effects from the introduced trait. Additionally, the Cry1A.105 and Cry2Ab2 proteins in MON 89034 are rapidly digested in simulated gastric fluids, making them unlikely to cause allergic reactions when consumed. A study evaluating MON 89034 corn in dairy cow rations found no significant differences in milk production compared to conventional corn. Sixteen lactating Holstein cows (684 ± 62.3 kg, 110 ± 21 DIM) were fed four diets containing: 1) non-transgenic control (DKC63-78), 2) MON 89034 (DKC63-78), 3) reference hybrid 1 (DKC61-42), and 4) reference hybrid 2 (DKC62-30) in a 4×4 Latin square design. Diets contained 36.4% corn silage and 16.3% corn grain. While dry matter intake (DMI) was higher for MON 89034 (26.6 ± 0.59 kg/d vs. 25.0–25.6 kg/d for controls), there were no differences in milk yield (36.8 ± 0.98 kg/d), fat (3.3 ± 0.10%), protein (3.01 ± 0.05%), MUN (14.01 ± 0.49 mg/dL), or 3.5% FCM (35.7 ± 1.07 kg/d). These results confirm that MON 89034 performs equivalently to conventional corn in dairy rations, supporting its safety and nutritional adequacy for livestock feed (see, Castillo-Lopez et al., 2014).

14(9). Name the food will be marketed under

The maize with events MON 89034 will be availed in the same formulations and combinations as the conventional maize, including whole grain, flours, processed corn products animal feed and whole

cobs. The varieties will bear the TELA maize Project brand as may be modified to differentiate them in accordance with the labelling and access to information regulations in Ethiopia.

15. Information required where the transaction relates to the placing of the modified organism as feed on the market

15(1). The potential for adverse health effects to humans and livestock, including exposure to toxins and anti-nutritional factors, as well as irritation and Allergenicity to humans;

The Cry1A.105 and Cry2Ab2 proteins, found in MON 89034, have been extensively evaluated and shown not to share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that could harm mammals. Bioinformatics tools, including FASTA sequence alignment and an amino acid sliding window search, confirmed the lack of any concerning similarities. Following FDA guidance, these proteins were concluded to pose "no concern" regarding human and animal health. As a result, food and feed products containing MON 89034 are considered safe for consumption (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

The safety assessment of MON 89034, based on its substantial equivalence to conventional maize, indicates no intrinsic hazards. Extensive testing, including animal feeding studies, has shown no adverse effects even at doses much higher than those expected in normal consumption. The risk analysis for food and feed use confirms that the consumption of MON 89034 or its derived products presents negligible risks, comparable to conventional maize products. As such, no specific risk management measures are needed, and post-market monitoring of MON 89034 in food, feed, or processing is unnecessary.

15(2). The potential to carry over of any novel product to manure and the potential impact of this to carry over;

Within consumed material including grain and forage, the proteins easily breakdown in gastric fluid and are not transferred to manure. The possibility of gene transfer through manure is nil since the genetic material is not transferrable though manure. The safety of the proteins and history of safe use has been demonstrated (EPA, 2010; EFSA, 2008).

15(3). Nutritional data which includes a comparison of the composition of the novel feed ingredient to that of the same feed ingredient derived from an appropriate counterpart;

Nutritional similarity between the modified maize and its conventional counterpart has been demonstrated indicating that neither the nutritional value nor the safety of the maize has been altered by the inserted genes. Detailed data is provided in Section 2, Part I, of this application. Safety assessment has been discussed in section 7(2) (b) and 7(2) (k) (Drury et al., 2008).

15(4). Dietary exposure;

Studies on the safety of foods derived from genetically modified (GM) maize containing event MON 89034 have shown that the insect-resistant proteins Cry1Ab.105 and Cry2Ab2, derived from the bacterium Bacillus thuringiensis (Bt), do not pose toxicity risks. Safety tests conducted on mice, with doses much higher than typical human consumption, revealed no evidence of toxicity. This suggests

that these proteins are safe at levels far exceeding what humans and animals would normally ingest from GM maize products (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

Regulatory bodies, after reviewing these studies, have concluded that foods made from MON 89034 maize, including its derivatives like corn-based products, are safe for both adults and children. There is reasonable certainty that these GM foods will not negatively affect human health, reinforcing the idea that they are as safe as conventional foods. Overall, the research supports that maize with MON 89034 and its proteins present no health risks to consumers.

15(5). Toxicology data such as endogenous toxins or newly expressed material;

The potential toxicity of the two cry proteins is evaluated based on several key factors: the protein's history of safe use, its lack of structural similarity to known toxins or biologically active proteins that could harm humans or animals, and the absence of acute toxic effects in mammals. Additionally, the genes for these proteins were sourced from organisms regarded as safe, and both proteins have a long history of safe consumption in food and animal feed. Importantly, these proteins do not resemble known toxins, and the data supports their safety for human and animal consumption (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

The safety of the two cry proteins is further reinforced by the long track record of maize containing the Mon 89034 event, which has been safely used worldwide in commercial agriculture. Additionally, the safety of the proteins is supported by over 50 years of experience with Bt microbial formulations, which have been safely used as biopesticides on various crops. This extensive history of safe use underscores the conclusion that these proteins are not toxic and pose no significant risk to human or animal health.

15(6). Allergenicity data

The assessment of the allergenic potential for maize with event MON 89034 was conducted according to the Codex Alimentarius Commission's guidelines for evaluating the allergenicity of novel proteins (Codex Alimentarius Commission, 2003). The guidelines suggest that a protein is unlikely to be allergenic if it originates from a non-allergenic source, constitutes a small proportion of the total protein in the grain, lacks structural similarity to known allergens, and is easily digested in simulated digestive fluids. Since maize is not a common allergenic food and food allergies to maize are rare, mainly occurring in specific populations, there is no expectation that maize with event MON 89034 will increase maize intake or exposure. Consequently, the overexpression of any non-allergenic endogenous proteins in this maize variety is not expected to affect the overall allergenicity of the plant or pose an increased allergy risk to consumers (Castillo-Lopez et al., 2014; EPA, 2010; EFSA, 2008).

15(7). Laboratory animal/livestock feeding trials

Dietary intakes of the Cry1Ab proteins for broiler chicken, young pig, finishing pig and lactating cows were estimated using the daily maize grain consumption and the highest level of the protein (0.69 µg/g fwt) in grain. The highest expression levels were used since there are situations where farmers grow maize for feeding their livestock directly on farm. For the lactating dairy cow, dietary intake was estimated based on the daily maize grain and forage (maize silage) consumption. There

was negligible risk to poultry and livestock from dietary exposure to animal feed containing maize with events MON 89034 (Castillo-Lopez et al., 2014; Drury et al., 2008).

16. Socio-Economic Considerations

Socio-economic Perspectives for Application for Deliberate Release of Maize with Event MON 89034 in Ethiopia in accordance with the Biosafety Amendment Proclamation No. 896/2015

1. Introduction

Maize is the second most important staple food in Ethiopia (after Teff), along with rice, beans, sorghum and millet. About 10 million households grow maize in Ethiopia (CSA, 2019). Currently, in Sub-Sahara Africa (SSA), Ethiopia has the fifth largest area devoted to maize. It is second only to South Africa in yield; and third after South Africa and Nigeria in production. Maize currently occupies about 2 million hectares with an average yield of over 3 MT/ha. It is a multipurpose crop, as the grain can be used as food in several forms such as whole-maize foods, wet-ground maize foods, snacks and bread, maize sourdough and dumplings, porridges and beverages, with grains and stovers also used as feed for livestock (Tostão et al., 2010).
Maize is both a subsistence and commercial crop. It is consumed as food and used for feed, seed or industrial materials. Maize production is affected by a wide range of constraints including the use of unimproved technologies, erratic climatic conditions causing frequent drought events, and high incidence of pests such as stem borers. Because of the importance of maize in Ethiopia, addressing these constraints to enhance maize productivity will contribute to improved farmer wellbeing and contribute to food and nutrition security in the country.

2. The problem

Impact of insect-pests on maize production in Ethiopia

Insect pests pose a significant threat to agriculture across Africa, causing substantial economic losses and impacting food security. Specifically, pests such as the fall armyworm (FAW) and various stem borer species are major constraints to maize production. These pests can lead to yield losses as high as 20% globally, with some areas suffering up to 73% damage depending on agro-ecological conditions. In Ethiopia, stem borers alone contribute to yield reductions of up to 15%, while in neighboring Kenya, losses from these pests reach over 400,000 MT annually, valued at more than USD 90 million (Oerke, 2002; Cugala et al., 2003; Seshu et al., 1990).

Similarly, the fall armyworm (FAW, Spodoptera frugiperda) has caused severe damage to maize production in Ethiopia since its invasion in 2017, leading to significant yield losses. According to the study, FAW infestation rates ranged from 1.7% to 34.5% in the first three years, from 2017-2020 with regional variations showing devastating impacts, across the country including Gambella (51.18% yield loss), Afar (59.22%), Oromia (25.2%), SNNPRS (23.1%), Amhara (18.26%), Tigray (9.8%), Ethio-Somalia (99.13%) and Benshangul Gumuz (65.8%) (Wondimu et al., 2021). The pest's rapid spread and resistance to synthetic pesticides exacerbated food insecurity, with smallholder farmers bearing the brunt due to limited control options.

The economic and health impacts of FAW are profound, with synthetic pesticide use costing farmers 3.6 million to 8.8 million US dollars (Wondimu et al., 2021). The study underscores the urgent need

for integrated pest management (IPM) strategies, including resistant crop varieties to mitigate FAW's threat to Ethiopia's maize-dependent food security (Wondimu et al., 2021).

Efforts to control these pests have included Integrated Pest Management (IPM), chemical treatments, and cultural control methods. However, these strategies face significant challenges, including impracticality for farmers and the adverse effects of pesticide use, such as resistance development and harm to non-target species. Moreover, insecticides often fail to effectively target pests like stem borers, which reside within plant tissues, making control difficult. The overuse of insecticides and the associated health risks, including occupational exposure, further complicate their use in pest management (Van den Berg & Nur, 1998; Bhat et al., 2012; Varela et al., 2003).

The limitations of conventional pest control methods have spurred interest in biotechnology as a potential solution. Advances in genetic engineering have made it possible to develop crops resistant to pests like stem borers and drought, offering hope for more sustainable agricultural practices. This includes the introduction of genes encoding proteins that protect plants from lepidopteran pests, which could significantly reduce dependency on chemical insecticides and enhance food security (Beemer, 2018; Glover, 2001; Brookes & Barfoot, 2018).

3. Alternative solution to address problem of drought-stress and insect-pests

GM crops—also called genetically engineered crops or biotechnology crops—have been repeatedly proven safe and successfully used to improve agricultural productivity around the world. Therefore, bioengineered crops offer African farmers an alternative and affordable insect pest control practice to chemical sprays (Hofte et al., 1989; Sims, 1997; Huesing et al., 2004). The need has never been greater for GM crops, which are bred to have traits beneficial to farmers, consumers and the environment.

The Bt maize was developed to allow plants to protect themselves from insect pests thus reducing the frequency of expensive chemical sprays. Bt crystal proteins are highly specific to certain caterpillars and do not impact other non-target organisms (Hofte et al., 1989; MacIntosh et al., 1989; Sims, 1997), unlike conventional synthetic insecticides; many of which kill a wide spectrum of both targeted and non-targeted (often beneficial) arthropods thereby affecting biodiversity. Maize that is genetically improved for insect protection is less prone to insect damage and yields much higher than their unmodified counterparts. In addition, such maize will require less or even no insecticide applications and constitutes a deliberate effort towards use of new alternative agricultural technologies that pose less potential risk to humans and the environment by maintaining a low carbon footprint.

The BPPD acknowledges that MON 89034 corn, expressing both Cry1A.105 and Cry2Ab2 proteins, offers broader lepidopteran pest protection than MON 810 (Cry1Ab only). In addition to targeting European corn borer (ECB), it effectively controls secondary pests like corn earworm (CEW), fall armyworm (FAW), and black cutworm (BCW). While this dual-protein trait may reduce pesticide use for secondary pests, most growers already avoid treating non-soil pests. Yield performance matches other Bt corn varieties, but under high pest pressure, the dual-toxin action could enhance protection and potentially increase yields compared to single-toxin Bt corn (EPA, 2010)

4. Potential benefits of insect-protected GM maize

GM crops, which were first commercially planted in the United States in 1996, have been widely

adopted across the globe. By 2017, over 189 million hectares in 67 countries were devoted to biotech crops, marking the fastest adoption rate of any agricultural technology in human history (James, 2018). Over 18 million farmers have benefitted from biotech crops, with 90% of them being small, resource-poor farmers (James, 2018). These crops offer significant economic benefits, particularly in developing countries. For example, for each additional dollar invested in GM crop seeds, farmers in developing countries see a return of $5.06, compared to $2.70 in developed countries (Brookes and Barfoot, 2018). The major advantage of GM crops, such as insect- and disease-resistant varieties, lies in their ability to help farmers protect crops more effectively and consistently. Studies by the International Food Policy Research Institute (IFPRI) and other projects highlight the potential economic benefits of adopting GM crops in Africa, particularly the stacked drought- and insect-resistant (TELA®) maize varieties. If adopted in Ethiopia and Uganda, these varieties could provide significant economic gains estimated at 2.29 billion PPP dollars for Ethiopia and 5.57 billion PPP dollars for Uganda (Table 1). The analysis also points out the risks associated with delays in policy approval for GM crops. For instance, Ethiopia could lose over 700 million PPP dollars if approval for the TELA® maize is delayed by five years (Figure 4), underlining the urgency of timely regulatory processes to maximize the benefits of biotechnology.

Table 1: Total net benefits of adopting biotech crops in millions international purchasing power parity (PPP) dollars[1]

ETHIOPIA	GHANA	TANZANIA	UGANDA
Insect and drought resistant maize	**Nitrogen efficient rice**	**Insect and drought resistant maize**	**Wilt resistant banana**
2,286	**13**	**5,573**	**2,336**
NIGERIA	**GHANA**	**TANZANIA**	**UGANDA**
***Maruca* resistant cowpea**	***Maruca* resistant cowpea**	**Brown streak resistant cassava**	**Brown streak resistant cassava**
810	**79**	**553**	**1,861**

[1]The benefits are calculated over a simulation period that differ by country and range between 25 to 35 years.

PPP is a metric used in macroeconomic analysis, as exchange rate for the number of units of a country's currency (e.g. Ethiopian Birr) required to buy the same amounts of goods and services in the domestic market as U.S. dollar would buy in the United States.

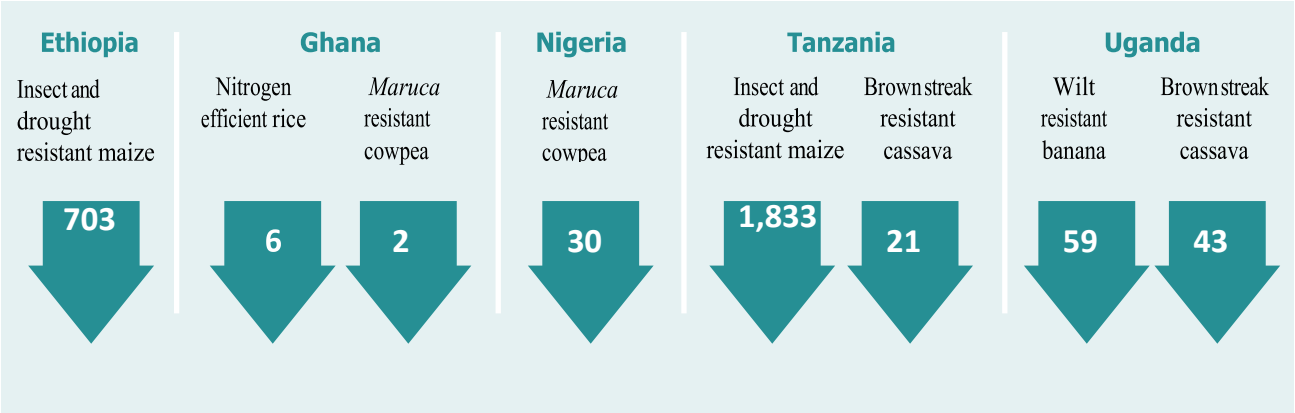


Figure 4: Losses due to a 5-year policy delay in millions international PPP dollars.

Source: IPRI, BioRAPP Report, 2020: African economies would benefit from biotech crops.

The global socio-economic and environmental impacts of biotech crops, which have been commercially planted since 1996, have been studied extensively, showing positive contributions on farm-level economics and environmental effects. These studies highlight the benefits of reduced insecticide use and contributions to reducing greenhouse gas emissions (Brookes et al., 2006; Brookes et al., 2008; Brookes et al., 2010; Hutchison, 2010; Brookes et al., 2011; Brookes et al., 2011; Mendez, 2011; Brookes et al., 2012; Brookes et al., 2014; Rice, 2014; Brookes and Barfoot, 2018). The introduction of insect-protected (Bt) crops has been instrumental in reducing production risks, especially by minimizing concerns over significant pest damage. This benefit is especially crucial for farmers in remote regions lacking access to pesticides, where crop losses due to pests can be as high as 60% (Brookes et al., 2010; Brookes et al., 2011). In these areas, GM crops with insect protection provide real economic advantages through lower production costs, improved yields, and simplified crop management.

The quality of crops has also improved due to the control of pests such as stem borers, which reduces the risk of mycotoxin contamination, a serious health hazard. Maize, which is particularly susceptible to aflatoxin contamination, benefits significantly from this protection, especially during periods of moisture stress when insect damage is prevalent. Pest-damaged grains are more prone to contamination during storage, which can lead to serious health issues such as aflatoxicosis, as seen in a 2004/05 outbreak in Kenya (Folcher et al., 2010). Research consistently shows that GM insect-resistant maize exhibits lower levels of mycotoxins compared to conventional maize, thus enhancing grain quality (Brookes et al., 2008). In the Philippines, farmers growing GM insect-protected maize have been able to command a price premium due to improved grain quality (Yorobe, 2004).

GM crops also save labor time, providing a significant convenience benefit. Farmers spend less time scouting for pests or spraying chemicals, allowing them to focus on other critical tasks such as weed control or increasing their acreage, which ultimately boosts production. The time saved can also be invested in other income-generating activities, improving the economic situation of smallholder farmers. Furthermore, the reduced need for pesticide use leads to improved health and safety for farmers and workers. In rural areas, particularly in developing countries like Ethiopia, exposure to pesticides is a significant health risk due to inadequate storage, lack of protective gear, and improper handling during spraying (Weisenburger, 1993; WHO, 2008; Pennsylvania State University, 2009). By reducing pesticide use, GM crops mitigate these health risks, improving the safety of farm workers.

The environmental benefits of Bt crops are also noteworthy, particularly in terms of reducing the negative impact of conventional chemical insecticides. Bt crops contribute to the reduction of chemical pollution, including the contamination of groundwater and the environmental hazards associated with disposing of pesticide containers (Zilberman et al., 2007). In addition to these benefits, the combined use of drought-tolerant (DT) and Bt maize hybrids offers a significant advantage for farmers in regions like Ethiopia. These hybrids not only protect against insect pests but also help farmers cope with drought conditions, positioning them to gain from these combined benefits in the near future.

5. Additional information on socioeconomic considerations

Futher information on how adoption of transgenic maize by growers will impact on food security and sustainability, access to the technology, income to farmers, cost of seeds and other inputs, co-existence, trade implications, benefits and freedom of choice, biosafety stewardship is outlined below.

5.1. Access to the technology, cost of seeds and other inputs

The drought tolerant and insect protection (Bt) traits used by the Project were accessed royalty-free for smallholder farmers in the Sub-Saharan Africa, implying that the there will be no additional payment of technology fee. Therefore, the seed companies or seed distrubutors that will be licensed to commercialize the products, will sell to farmers at the prevailing market prices for maize seed without charging farmers additional costs for the technology. The application in itself does not alter supply balance nor become different from how farmers access maize varieties in Ethiopia.

5.2 Income to farmers

Economic Benefits of MON 89034 Corn

MON 89034 corn delivers significant economic advantages through its expanded pest protection, improved grain quality, and cost reductions. The hybrid's dual-protein trait provides broader defense against lepidopteran pests including fall armyworm (FAW) and corn earworm (CEW), leading to higher yields and superior grain quality. Additionally, it reduces mycotoxin contamination, enhancing both crop value and food safety, while offering more effective corn borer control than single-protein Bt varieties. Growers also benefit from decreased pesticide expenses due to reduced chemical applications.

Table Economic Impact of MON89034

Benefit	Per Acre Benefit ($)	Total Benefit ($ Millions)
Yield increase	13.59 (-3.67 – 48.76)	217 (-59 – 780)
Pesticide reduction	1.99 (1.00 – 2.98)	32 (16 – 48)
Mycotoxin reduction	1.98 (0.52 – 7.12)	32 (8.3 – 114)

The technology's combined benefits create substantial value across all production scales, particularly in regions with significant lepidopteran pressure. The table above quantifies the potential economic gains, demonstrating MON 89034's advantage over conventional and single-trait Bt corn varieties.

5.3 Co-existence

When the safety of the stacked events has been demonstrated, the segregation of GM grain from non-GMO grain will not arise. The practice world-wide is to consider transgenic maize that has been evaluated for risk according to the Codex Guidelines and in comformity to the Cartagena Protocol to be as safe as its conventional counterpart. This is therefore, not treated as a socioeconomic consideration by the applicant.

Regarding seed production for which pure certified seed will be required from time to time, the Project notes that there is an elaborate seed production isolation distance established for all maize varieties and not just trangenic maize. These will apply to ensure farmers make choices on which seed to plant. This is also therefore, not a socioeconomic consideration.

The statement that "the transformed maize will not replace existing fields of conventional maize" does not imply the establishment of new, separate growing sites for MON89034. Instead, it reflects a coexistence strategy where genetically modified (GM) and conventional maize are grown in the same regions but managed to minimize cross-pollination and maintain supply chain segregation. MON89034 can be planted near conventional maize if it is produced for grain, but physical separation (e.g., 200–400 meters) or temporal isolation (staggered planting dates) is used to limit pollen flow. These measures align with guidelines from the OECD and EFSA to keep GM content in non-GM crops below labeling thresholds (e.g., 0.9% in the EU). Farmers growing MON89034 must adhere to contractual agreements with seed companies or buyers, ensuring GM and non-GM crops are kept separate during planting, harvesting, storage, and transport. This includes dedicated equipment and cleaning protocols to avoid mixing. Unlike some crops requiring designated GM-free zones, MON 89034 is integrated into existing maize-growing areas. It is possible that GM cultivation can be grown in any farm without displacing conventional fields, provided coexistence protocols are followed. For MON89034, no new sites are mandated; existing farmland is used, with coexistence managed through best practices rather than geographic exclusion.

5.4 Trade implications

In the application, the applicants have demonstrated that the transgenic maize is grown widely in many countries. Besides, Ethiopia's exports little or no grain maize. When the exports happen, it is mainly to neighboring Kenya which is already conducting national performance trials of the insect protected (TELA®) maize varieties. Trade is therefore, not considered to be a socioeconomic factor. The choice of markets that may not want GM maize will warrant farmers selling to such markets to produce non-GM maize, which will be available. The transformed maize will not replace existing fields of conventional maize.

The trade implications of GM maize production are significant, as it influences global agricultural markets, trade policies, and international relations. Countries that adopt GM maize often benefit from higher yields and reduced production costs, enhancing their export competitiveness, particularly in markets where GM crops are accepted like Kenya. There is diverse markets, including other African nations (e.g., Sudan, South Africa) and international buyers, while ensuring compliance with each region's GMO policies and labeling standards.

5.5 Biosafety and stewardship

It is considered that the entire prescribed form is a biosafety assessment platform. Therefore, the question of biosafety is addressed in the application form appropriately. Elaborate stewardship and monitoring plans have been provided in Section 12 of the Application Form.
All other necessary information to aid in decision making regarding socio-economic considerations is provided in the Application Dossier.

6. Conclusion

Biotechnology has made significant contributions to agriculture, offering agronomic traits that help mitigate production challenges for farmers globally. By 2018, up to 58.9 million hectares of genetically modified (GM) maize had been planted worldwide, boosting productivity and profitability. GM technology has shown positive socio-economic and environmental impacts, with reductions in pesticide use by 37%, yield increases of 22%, and farmer profit boosts of 68%. These

benefits are particularly pronounced for insect-resistant crops and are more notable in developing countries like South Africa, where GM maize, soybean, and cotton led to over $1 billion in increased farm income between 1998 and 2012 (James, 2018; Gouse et al., 2005).

Ethiopia, a major agricultural economy in Africa, could see similar benefits from the adoption of GM crops, particularly maize. With smallholder farmers growing more than 75% of Ethiopia's maize, the approval of GM maize varieties such as TELA® maize, which offers water-use efficiency and resistance to pests, could drastically improve productivity. If commercialized, TELA® maize could provide substantial benefits, particularly for poorer households that spend a significant portion of their income on food. This biotechnology could contribute to poverty reduction by increasing maize yields, lowering food prices, and ultimately benefiting farmers and consumers alike in Ethiopia (James, 2018).

Declaration of correctness of Information

I certify that the above information is true to the best of my Knowledge

Principal Investigator

Name: Tesfaye Disasa, PhD

Signature: 

Date: 06 April 2025

References

1. Ahmad, A., G.E. Wilde, and K.Y. Zhu. 2005. Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. Environ. Entomol. 34:385-394.
2. Al-Deeb, M.A., G.E. Wilde and R.A. Higgins. 2001. No effect of *Bacillus thuringiensis* corn and Bacillus thuringiensis on the Predator Orius insidiosus (Hemiptera: Anthocoridae). Environ. Entomol. 30: 625-629.
3. Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons.
4. Armstrong C.L. and Phillips R.L. (1988). Genetic and cytogenetic variation in plant regenerated from organogenic and friable, embryogenetic tissue culture of maize. Crop Sci 28: 363-369
5. Aronson, A.I. and Y. Shai. 2001. Why Bacillus thuringiensis insecticidal toxins are so effective: unique features of their mode of action. FEMS Microbiol. Lett. 195: 1-8.
6. Barker, R. F., Idler, K. B., Thompson, D. V. and Kemp, J. D. (1983) Nucleotide sequence of the T-DNA region from the Agrobacterium tumefaciens octapine Ti Plasmid pTi15955, Plant. Mol. Biol., 2, 335-350.
7. Beadle, G.W. 1978. Teosinte and the origin of maize. Maize breeding and genetics. Section 2. Evolution.: 113-128.
8. Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B. and Schaller, H. (1982). Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. Gene 19: 327-36.
9. Beemer, L. (2018). Fall armyworm a serious threat to sub-Saharan African food security in 2018. https://www.agribusinessglobal.com/markets/africa-middle-east/fall-armyworm-a-serious-threat-to-sub-sarahan-african-food-security-in-2018/ (Accessed 6 September 2020).

10. Bevan, M., Barnes, W.M. and Chilton, M.D. (1983) Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Res., 11, 369-385.
11. Bhat, Z.H. and Z.A. Baba. 2012. Efficacy of different insecticides against maize stem borer, Chile patella's (Swine) and maize Aphid, Rhopalosiphum maids (Fitch) infesting maize. Pak. Entomol. 29: 73-76.
12. Brookes, G. and P. Barfoot. 2006. GM Crops: The First Ten Years - Global Socio-Economic and Environmental Impacts. International Service for the Acquisition of Agri-biotech Applications (ISAAA) Brief No. 36. ISAAA: Ithaca, New York: 97.
13. Brookes, G. and P. Barfoot. 2008. Global impact of biotech crops: socio-economic and environmental effects 1996-2006. Available on the World Wide Web: http://www.agbioforum.org. Agbioforum 11: 21-38.
14. Brookes, G. and P. Barfoot. 2010. GM crops: global socio-economic and environmental impacts 1996-2008. PG Economics Ltd, Dorchester, UK.
15. Brookes, G. and P. Barfoot. 2011. Global impact of biotech crops: environmental effects 1996-2009. GM Crops 2: 34-49.
16. Brookes, G. and P. Barfoot. 2012. GM crops: global socio-economic and environmental impacts 1996-2010. PG Economics Ltd, Dorchester, UK.
17. Brookes, G. and P. Barfoot. 2014. GM crops: global socio-economic and environmental impacts 1996-2012. PG Economics Ltd, Dorchester, UK.
18. Brookes, G. and P. Barfoot. 2018. Farm income and production impacts of using GM crop technology 1996-2016. GM Crops and Food 9(2) 59-89.
19. Brown, S.M. and Santino, C.G. (1995) Enhanced expression in plants. United States Patent, Patent number 5.424.412
20. Cannon, R. J. C. (1993). Prospects and progress for Bacillus thuringiensis-based pesticides, Pesticide Science, 37, 331-335.
21. Codex Alimentarius Commission. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Codex CAC/GL 452003: 1-13.
22. Crickmore, N., D.R. Zeigler, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, A. Bravo and D.H. Dean. 2005. Bacillus thuringiensis toxin nomenclature. http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/.
23. Crickmore, N., D.R. Zeigler, J. Feitelson, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum and D.H. Dean. 1998. Revision of the nomenclature for the Bacillus thuringiensis pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 807-+
24. CSA (2019). Ethiopian Agricultural Sample Survey 2018/19. Report on Area, Crop and livestock
25. Cugala, D., C. Omwega, C. Ogol and Santos, L. 2003. Establishment of Cotesia flavipes population in Mozambique. African Crop Science, 6: 241-245.
26. De Block M, Herrera-Estrella L, Van Montagu M, Schell J and Zambryski P, 1984. Expression of foreign genes in regenerated plants and in their progeny. EMBO J., 3, 1681-1689.
27. De Schrijver, A. Devos Y., Van den Bulckea, M. Cadot P., De Loose, M., Reheul, D., Sneyersa, M. (2007). Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science & Technology. 18, Issue 2, February 2007, Pp 101–109
28. Depicker, A., Stachel, S., Dhaese, P., Zambryski, P. and Goodman, H. M. (1982) Nopaline synthase: transcript mapping and DNA sequence, J. Mol. Appl. Genet. 1:561-573.
29. Devos, Y., D. Reheul and A. De Schrijver. 2005. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross fertilization. Environ. Biosatety Res. 4: 71-87.
30. Dhaese, P., De Greve, H., Gielen, J., Seurinck, J., Van Montague, M. and Schell, J. (1983). Identification of sequences involved in polyadenylation of higher plant nuclear transcripts using Agrobacterium T-DNA genes as models, The EMBO Journal 2:419-426.
31. Donegan, K.K., D.L. Schaller, J.K. Stone, L.M. Ganio, G. Reed, P.B. Hamm, and R.J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the Bacillus thuringiensis var. tenebrionis endotoxin. Transgenic Res. 5:25-35.

32. Donovan, W. P. 1991. CryIIB crystal protein gene from Bacillus thuringiensis. United States Patent No. 5,073,632.
33. Dubelman, S., B.R. Ayden, B.M. Bader, C.R. Brown, C. Jiang, and D. Vlachos. 2005. Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environ. Entomol. 34:915-921.
34. Dulmage, H.T. 1981. Insecticidal activity of isolates of Bacillus thuringiensis and their potential for pest control. Microbial control of pests and plant diseases 1970-1980. Burger, H.D. p. 193-222
35. Elrine Strydom, Annemie Erasmus, Stephanus Friis, Jacques Magson, Srinivas Parimi, Samuel Martinelli, Graham P Head, Hannalene du Plessis and Johnnie van den Berg, 2024. Incipient resistance of the African maizestemborer, Busseola fusca (*Lepidoptera:Noctuidae*) to Cry2Ab2 maize in South Africa, Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry
36. Emmerling, M., D. Chandler and M. Sandeman. 2001. Molecular cloning of three cDMAs encoding aminopeptidases from the midgut of Helicoverpa punctigera, the Australian native budworm. Insect Biochem. Mol. Biol. 31: 899-907.
37. English L., and Slatin, S. 1992. Endotoxins form cation-selective channels in planar lipid bilayers. Biochemical and Biophysical Research Communications 169(2):765-72
38. EPA. (1988). Guidance for the reregistration of pesticide products containing Bacillus thuringiensis as the active ingredient. NTIS PB 89-164198.
39. Federici, B. 2002. Case study: Bt crops-a novel mode of insect resistance. Pages 164-200 in Genetically Modified Crops: Assessing Safety. Atherton, K. (Ed.), Taylor & Francis Group, London.
40. Folcher, L., M. Delos, E. Marengue, M. Jarry, A. Weissenberger, N. Eychenne and R.-R. C. 2010. Lower mycotoxin levels in Bt maize grain. Agron. Sustain. Dev. 30: 711-719.
41. Fraley, R. T., Rogers, S. G., Horsch, R. B., Sanders, P. R., Flick, J. S., Adams, S. P., Bittner, M. L., Brand, L. A., Fink, C. L., Fry, J. S., Galluppi, G. R., Goldberg, S. B., Hoffmann, N. L. and Woo, S. C. (1983). Expression of bacterial genes in plant cells, Proc. Natl. Acad. Sci. USA, 80, 4803-4807.
42. Fuchs, R.L., J.E. Ream, B.G. Hammond, M.W. Naylor, R.M. Leimgruber, and S.A. Berberich. 1993b. Safety assessment of the neomycin phosphotransferase II (NPTII) protein. Bio/Technol. 11:1543-1547
43. Garner, K., S. Hiremath, K. Lethoma and A.P. Valaitis. 1999. Cloning and complete sequence characterization of two gypsy moth aminopeptidase-N cDNAs, including the receptor for Bacillus thuringiensis Cry1Ac toxin. Insect Biochem. Mol. Biol. 29: 527-535.
44. Glover, D. 2001. Modern Biotechnology and Developing World Agriculture. University of Sussex; Brighton, United Kingdom; Institute of Development Studies.
45. Goodman M.M, 1988. The history and evolution of maize. CRC Crital Rev. Plant Sciences, 7, 197-220.
46. Gouse, M., C. Pray, J.F. Kirsten and D. Schimmelpfennig. 2005. A GM subsistence crop in Africa: the case of Bt white maize in South Africa. International Journal of Biotechnology 7: 84-94.
47. Hallauer, A.R. 1995. Potential for outcrossing and weediness of genetically modified insect protected corn.
48. Halsey, M.E., K.M. Remund, C.A. Davis, M. Qualls, P.J. Eppard and S.A. Berberich. 2005. Isolation of maize from pollen-mediated gene flow by time and distance. Crop Sci. 45: 2172-2185.
49. Hofte, H. and H.R. Whiteley. 1989. Insecticidal crystal proteins of Bacillus thuringiensis. Microbiology Review 53: 242- 255.
50. Horsch RB, Fraley RT, Rogers SG, Sanders PR, Lloyd A and Hoffmann N, 1984. Inheritance of functional foreign genes in plants. Science, 223, 496-498.
51. Huesing, J. and L. English. 2004. The impact of Bt crops on the developing world. Available on the World Wide Web: http://www.agbioforum.org. AgBioForum 7: 84-95.
52. Hutchison, W.E.A. 2010. Area-wide suppression of European Corn Borer with Bt maize reaps savings to non-bt maize growers. Science 330: 222-225.
53. IPCS. (1999). Environmental Health Criteria 217: Bacillus thuringiensis, http://www.who.int/ipcs/ publications/ehc/en/ EHC217.
54. Ishida, Y., H. Saito, S. Ohta, Y. Hiei, T. Komari, and T. Kumashiro. 1996. High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens. Nat. Biotechnol. 14:745-750.

55. James, C. 2018. Global Status of Commercialized Biotech/GM Crops: 2018. ISAAA Brief No. 54. ISAAA: Ithaca, NY.
56. Kay, R., Chan, A., Daly, M. and McPherson, J. (1987) Duplication of CaMV 35S Promoter sequences creates a strong enhancer for plant genes. Science 236: 1299-1302.
57. Klausner, A. 1984. Microbial insect control. Biotechnology: 408-419.
58. Klein, T.M., E.D. Wolf, R. Wu and J.C. Sanford. 1987. High-velocity microprojectiles for delivering nucleic acids into living cells. Nature 327: 70-73.
59. Liener, I.E. (1994) Implications of Antinutritional Components in Soybean Foods. Critical Reviews in Food Science and Nutrition, 34, 31-67.
60. Luna, V. S., Figueroa, J.M., Baltazar, B.M., Gomez, R.L., Townsend R. and Schoper, J.B. (2001). Maize Pollen Longevity and Distance Isolation Requirements for Effective Pollen Control. Crop Sci.41: 1551-1557.
61. Ma, B.L., K.D. Subedi and L.M. Reid. 2004. Crop ecology, management & quality - extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. Crop Sci 44: 1273-1282.
62. MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff and R.L. Fuchs. 1990. Specificity and efficacy of purified Bacillus thuringiensis proteins against agronomically important insects. J. Invertebr. Pathol. 56: 258-266.
63. MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff and R.L. Fuchs. 1989. J. Invert. Path. 56: 258-266.
64. Martinez-Soriano, J.P.R., A.M. Bailey, J. Lara-Reyna and D.S. Leal-Klevezas. 2002. Transgenes in Mexican maize. Nature Biotechnology 20: 19-19. doi:10.1038/nbt0102-19a.
65. Matsuoka, M., Y. Kano-Murakami, Y. Tanaka, Y. Ozeki, and N. Yamamoto. 1987. Nucleotide sequence of the cDNA encoding the small subunit of ribulose-1,5 bisphosphate carboxylase from maize. J. Biochem. 102:673-676.
66. McElroy, D., Blowers, A. D., Jenes, B. and Wu, R. (1991). Construction of expression vectors based on the rice actin 1 (Act1) 5' region for use in monocot transformation. Molecular Genetics 231: 150-160.
67. McElwain, E. F., & Spiker, S. (1989). A wheat cDNA clone which is homologous to the 17 kd heat-shock protein gene family of soybean. *Nucleic acids research*, *17*(4), 1764.
68. McElwain, E. F., & Spiker, S. (1989). A wheat cDNA clone which is homologous to the 17 kd heat-shock protein gene family of soybean. *Nucleic acids research*, *17*(4), 1764.
69. Mendelsohn, M., J. Kough, Z. Vaituzis and K. Matthews. 2003. Are Bt crops safe? Nature Biotechnology 21: 1003-1009.
70. Mendez, K.E.A. 2011. Production cost analysis and use of pesticides in the transgenic and conventional crop in the valley of San Juan (Colombia). GM Crops 2: 163-168.
71. Muhyideen Oyekunle , R S Adamu , E Ndou , Y Beyene , M M Abdulmalik , S O Oikeh, 2023, Efficacy of drought-tolerant and insect-protected transgenic TELA® maize traits in Nigeria, *Transgenic Res* **32**, 169–178 (2023). https://doi.org/10.1007/s11248-023-00345-x
72. Noteborn, H.P. and H.A. Kuiper. 1994. Safety assessment strategies for genetically modified plant products: a case study of Bacillus thuringiensis-toxin tomato. Biosafety of foods derived by modern biotechnology, BATS.
73. Odell, J.T., Nagy, F. and Chua, N.H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313, 810-812
74. OECD, 2003. Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants. OECD, ENV/JM/MONO (2003)10, 1-49.
75. OECD. 2003b. Consensus Document on the Biology of Zea Mays Subsp. Mays (Maize). http://www.oecd.org/ OECD ENV/JM/MONO (2003) 11: 1-49.
76. Oerke, E.C. 2002. Crop losses due to pests in major crops. In Crop protection compendium 2002: Economic impact. Wallingford, UK: CAB International.
77. Pennsylvania State University. 2009. Potential Health effects of pesticides – Pesticide safety fact
78. Pleasants, J.M., R.L. Hellmich, G.P. Dively, M.K. Sears, D.E. Stanley-Horn, H.R. Mattila, J.E. Foster, T.L. Clark and G.D. Jones. 2001. Corn pollen deposition on milkweeds in and near cornfields. Proc. Natl. Acad. Sci. USA 98: 11919-11924.

79. Rice, M. 2014. Transgenic rootworm corn: assessing potential agronomic, economic and environmental benefits. Plant Health Progress 10.
80. Rogers, S.G. 2000. Promoter for transgenic plants. United States Patent No. 6,018,100.
81. Roh, J., J. Choi, M. Li, B. Jin and Y. Je. 2007. Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. Journal of Microbiology and Biotechnology 17: 547-559.
82. Romeis, J., M. Meissle and F. Bigler. 2006. Transgenic crops expressing Bacillus thuringiensis toxins and biological control. Nature Biotechnology 24: 63-71.
83. Sacchi VF, Parenti P, Hanozet GM, Giordana B, Lüthy P and Wolfersberger MG, 1986. Bacillus thuringiensis toxin inhibits K+-gradient-dependent amino acid transport across the brush border membrane of Pieris brassicae midgut cells. Febs Letters, 204, 213-218.
84. Sanchis, V. and D. Bourguet. 2008. Bacillus thuringiensis: applications in agriculture and insect resistance management. A review. Agronomy for Sustainable Development 28: 11-20.
85. Sanders, P.R., Lee, T.C., Groth, M.E., Astwood, J.D. and Fuchs, R.L. (1998). Safety assessment of insect-protected corn. In Biotechnology and Safety Assessment, 2nd ed; Thomas, J.A. Ed., Taylor and Francis, pp 241-256.
86. Sears, M.K., R.L. Hellmich, D.E. Stanley-Horn, K.S. Oberhauser, J.M. Pleasants, H.R. Mattila, B.D. Siegfried and G.P. Dively. 2001. Impact of Bt corn pollen on Monarch butterfly populations: a risk assessment. Proc. Natl. Acad. Sci. 98: 11937-11942.
87. Seshu, R.K.V. and W. P.T. 1990. A review of the yield loss in graminaceous crops caused by Chile spp. Insect Science and its Application 11: 563-569.
88. Shaw RH, 1988. Climate requirement. In: Corn and corn improvement - Agronomy Monograph. GF Sprague, JW Dudley. American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, Wisconsin, 609-638.
89. Sims, S.R. 1997. Host activity spectrum of the Cry2A Bacillus thuringiensis susb. kurstaki protein: Effects on Lepidoptera, Diptera, and non-target arthropods. Southwest Entomology 22: 395-404.
90. Sjoblad, R. D. Mcclintock J.T and Engler R. (1992). "Toxicological Consideration for Protein Components of Biological Pesticide Products", Regulatory Toxicology and Pharmacology 15: 3- 9
91. Smith B D. The Emergence of Agriculture. New York: Scientific American Library; 1998.
92. Tabashnik, B.E. 2008. Delaying insect resistance to transgenic crops. Proceedings of the National Academy of Sciences of the United States of America 105: 19029-19030. doi:10.1073/pnas.0810763106.
93. Tiedje, J.M., S. Asuming-Brempong, K. Nusslein, T.L. Marsh and S.J. Flynn. 1999. Opening the black box of soil microbial diversity. Appl. Soil Ecol. 13: 109-122.
94. Tostão et al., 2010. "Staple food prices in Mozambique", Prepared for the COMESA policy seminar on "Variation in staple food prices: Causes, consequence, and policy options", Maputo, Mozambique, 25-26 January 2010.
95. US EPA. 1998. Reregistration eligibility decision (RED). Prevention, Pesticides and Toxic Substances: 1-157.
96. US EPA. 2001. Biopesticides registration action document: Bacillus thuringiensis (Bt) plant incorporated protectants. U.S. Environmental Protection Agency: 1.
97. Van den Berg, J., Nur, A.F., 1998. Chemical Control. In Polaszek., A., 1998. (Ed.) African cereal stem borers: economic importance, taxonomy, natural enemies and control. CAB International, Wallingford, Oxon, UK, 530 pp.
98. Van Mellaert, H., J. Van Rie, C. Hofmann and A. Reynaerts. 1988. Insectidical crystal proteins from Bacillus thuringiensis: mode of action and expression in transgenic plants. Conference on biotechnology, Biological pesticides and novel plant-pest resistance for insect pest management.
99. Weisenburger, D.D. 1993. Human health effects of agrichemical use. Human Pathol 24 571 - 576.
100. Whiteley, H.R. and H.E. Schnepf. 1986. The molecular biology of parasporal crystal body formation in Bacillus thuringiensis. Annu Rev Microbiol 40: 549-576.
101. WHO, F. 2008. International code of conduct on the distribution and use of pesticides. Guidelines on management options for empty pesticide containers.

102. Widner WR, Whiteley HR. 1989. Two highly related insecticidal crystal proteins of Bacillus thuringiensis subsp. kurstaki possess different host range specificities. J Bacteriol 171:.
103. Wright, D., J. Marois, J. Rich, and R. Sprenkel. 2004. Field corn production guide. Document SS-AGR-85, Department of Agronomy, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available online at http://edis.ifas.ufl.edu/AG202 . Accessed September 19, 2006.
104. Yorobe, J. 2004. Economics impact of Bt corn in the Philippines. Paper presented to the 45th PAEDA Convention, Querzon City.
105.
106. Zhang, H., L. Feng, L. Xiao and X. Song. 2015. Impact of water content and temperature on the degradation of Cry1Ac protein in leaves and buds of Bt cotton in the soil. PLoS ONE 10: e115240. doi:
107. Zilberman, D., Ameden H and Q. M. 2007. The impact of agricultural biotechnology on yields, risks, and biodiversity in low-income countries. J. Dev. Stud. 43: 63-78.

New References

1. BPPD, 2007i. Review of non-target invertebrate studies for MON 89034 maize containing Cry1A.105 and Cry2Ab2 proteins. M. Hunter memo to S. Cerrelli, 12/14/2007.
2. BPPD, 2007j. Review of broiler, quail, soil fate, and endangered species data packages submitted in support of MON 89034 Bt corn, T. Milofsky to S. Cerrelli, 12/14/2007.
3. Castillo-Lopez, E., Clark, K. J., Paz, H. A., Ramirez, H. R., Klusmeyer, T. H., Hartnell, G. F., & Kononoff, P. J. (2014). Performance of dairy cows fed silage and grain produced from second-generation insect-protected (Bacillus thuringiensis) corn (MON 89034), compared with parental line corn or reference corn. Journal of dairy science, 97(6), 3832-3837.
4. Chumakov, M. I., Gusev, Y. S., Bogatyreva, N. V., & Sockolov, A. Y. (2019). Risks of pollen-mediated gene flow from genetically modified maize during co-cultivation with usual maize varieties. Biology Agricultural, 426.
5. Drury, S. M., Reynolds, T. L., Ridley, W. P., Bogdanova, N., Riordan, S., Nemeth, M. A., ... & Breeze, M. L. (2008). Composition of forage and grain from second-generation insect-protected corn MON 89034 is equivalent to that of conventional corn (Zea mays L.). Journal of Agricultural and Food Chemistry, 56(12), 4623-4630.
6. EFSA (European Food Safety Authority), 2008. Scientific Opinion– Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of insect-resistant genetically modified maize MON 89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2008; 6 (12):909, 30 pp. https://doi.org/10.2903/j.efsa.2009.909
7. EFSA Panel on Genetically Modified Organisms (GMO), Naegeli, H., Bresson, J. L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., ... & Raffaello, T. (2019). Assessment of genetically modified maize MON 89034 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-015). EFSA Journal, 17(11), e05845.
8. EFSA Panel on Genetically Modified Organisms (GMO), Naegeli, H., Bresson, J. L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., ... & Raffaello, T. (2019). Assessment of genetically modified maize MON 89034 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-015). EFSA Journal, 17(11), e05845.
9. EFSA Panel on Genetically Modified Organisms (GMO). (2010). Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034× 1507× MON 88017× 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto. EFSA Journal, 8(9), 1781.
10. EFSA Panel on Genetically Modified Organisms (GMO). (2010). Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034× 1507× MON 88017× 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto. EFSA Journal, 8(9), 1781.

11. EPA. (2010). Biopesticide Registration Action Document. Bacillus thuringiensis Cry1A. 105 and Cry2Ab2 Insecticidal Proteins and the Genetic Material Necessary for Their Production in Corn [PC Codes 006515 (Cry2Ab2), 006514 (Cry1A. 105)].
12. European Food Safety Authority (EFSA). (2008). Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal, 6(12), 909.
13. European Food Safety Authority (EFSA). (2008). Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal, 6(12), 909.
14. FSANZ (2008, June 4). Food derived from insect-protected corn line MON 89034. Application 595, Final Assessment Report. http://www.foodstandards.gov.au/standardsdevelopment/
15. Wondimu, A., Petros, B., Asfaw, Z., & Woldehawariat, Y. (2021). Prevalence and impacts of fall army worms (Spodoptera frugiperda) on maize (Zea mays) production and productivity in Ethiopia. ISABB Journal of Food and Agricultural Sciences, 10(2), 21-27.

Annex 1.

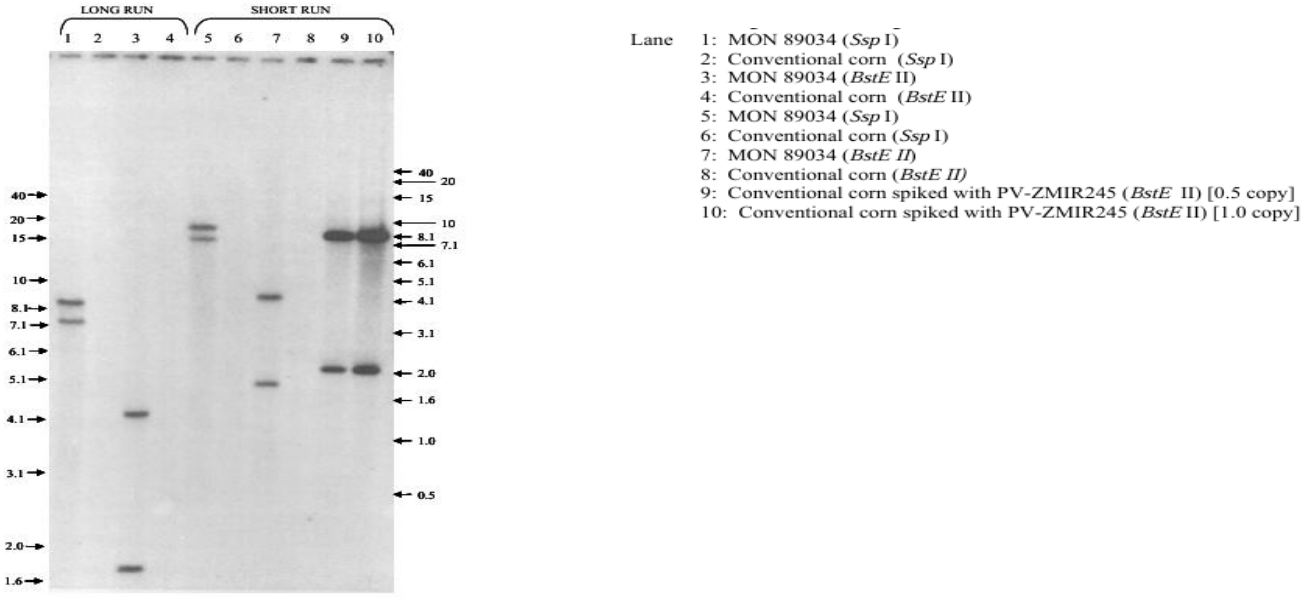
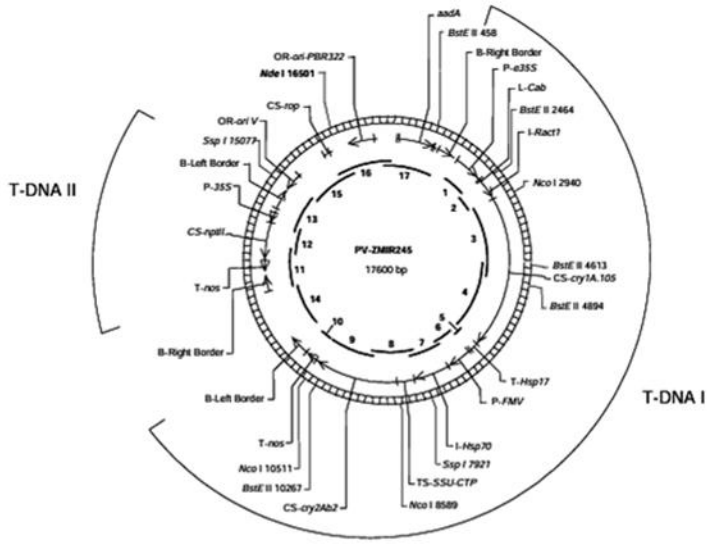


Figure 1 Shows the Southern blot analysis to determine the presence or absence of T-DNA II. Conventional corn control DNA digested with Ssp I (lanes 2 and 6) and BstE II (lanes 4 and 8) examined with overlapping probe spanning T-DNA II (probes 11, 12, and 13 showed no hybridization bands.



Probe Number	DNA Probe Description	Start Position (bp)	End Position (bp)	Total Length (~kb)
1	P-*e35SL*-*Cab*	1714	2447	0.7
2	I-*Ract1*	2427	2941	0.5
3	CS-*cry1A.105* probe 1	2942	4923	2.0
4	CS-*cry1A.105* probe 2	4726	6505	1.8
5	T-*Hsp17*	6490	6797	0.3
6	P-*FMV*	6755	7366	0.6
7	I-*Hsp70*	7347	8179	0.8
8	TS-*SSU-CTP*/CS-*cry2Ab2* probe 1	8173	9516	1.3
9	TS-*SSU-CTP*/CS-*cry2Ab2* probe 2	9296	10509	1.2
10	T-*nos*	10525	10778	0.3
11	T-DNA II probe 1	12458	13391	0.9
12	T-DNA II probe 2/CS-*nptII* probe	13256	14050	0.8
13	T-DNA II probe 3	13973	14916	0.9
14	Backbone 1	11287	12489	1.2
15	Backbone 2	14900	16511	1.6
16	Backbone 3	16289	136	1.4
17	Backbone 4	48	1261	1.2

Figure 2. Plasmid map of vector PV-ZMIR245 showing probes 1-17 used in Southern blot analyses

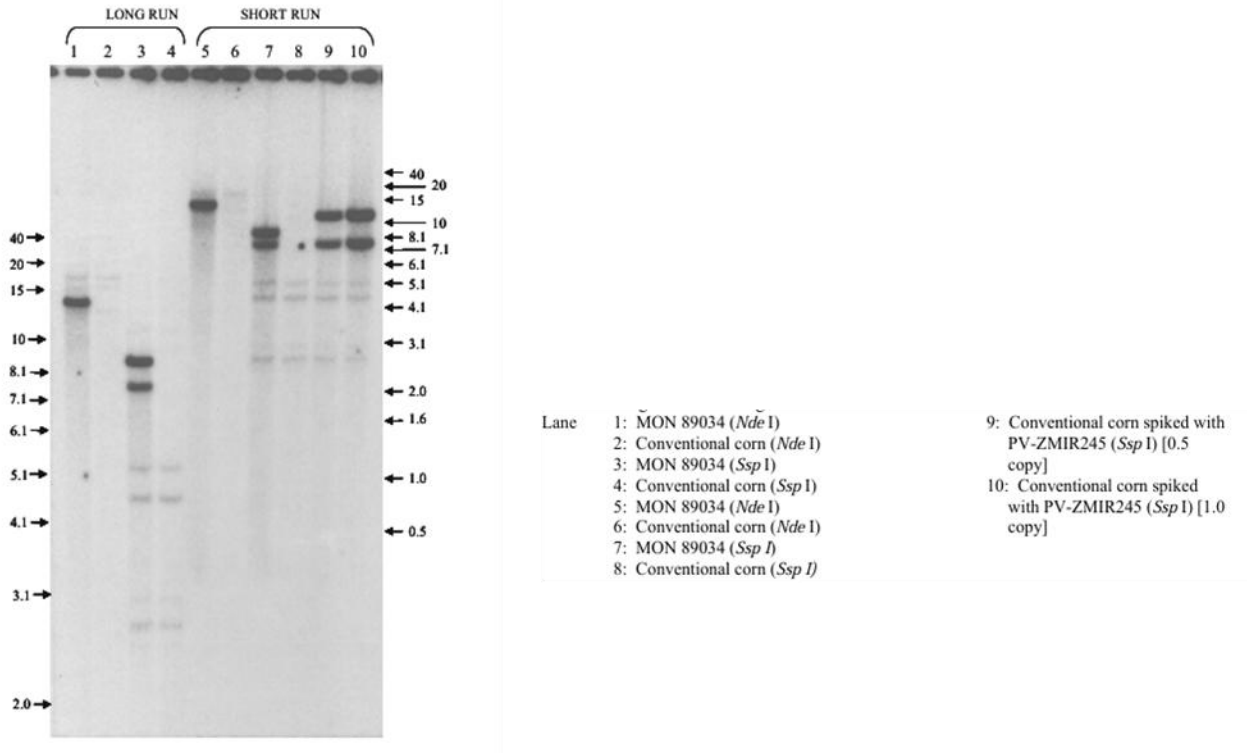


Figure 3. Southern blot analyses of MON 89034 insert and copy number analysis

Note: MON 89034 DNA digested with Nde I (lanes 1 and 5) produced a single unique band of approximately 13 kb in addition to the endogenous background hybridization observed in the conventional corn control DNA (lanes 2 and 6). This result confirms that MON 89034 contains one insert located on an approximately 13.0 kb Nde I restriction fragment.

Annex II

Table 1. Summary of Cry1A.105 Human Health Data

Study Type/Title	Summary	MRID #
Acute oral toxicity (OPPTS 870.1100)/ Acute Oral Toxicity	The Cry1A.105 test protein (2072 mg/kg body weight) was dosed by gavage as two doses separated by 4 hours (±20 minutes). The BSA protein control (1998	46694603

Study Type/Title	Summary	MRID #
Study in Mice with Cry1A.105 Protein	mg/kg body weight) was dosed using the same procedure as for the test protein group. The vehicle control group was dosed with carbonate bicarbonate with reduced glutathione. Body weight was recorded prior to fasting, prior to dosing, and on days 7 and 14. The test animals were observed for clinical signs of toxicity two times post-dosing and for 14 days. A general health/mortality check was done twice daily. All animals were necropsied. One male in the test protein group was moribund and sacrificed on day 1 due to a mechanical dosing error, which resulted in a perforated esophagus. All other mice survived the study. There were no significant differences in body weight or body weight change among the three groups during the study. The oral LD50 for males, females, and combined mice was greater than 2072 mg/kg. This places Cry1A.105 Protein in TOXICITY CATEGORY III due to dose amounts only; no signs of toxicity were observed. **Classification: ACCEPTABLE**	
Amino acid sequence comparison/ Bioinformatics Analysis of the Cry1A.105 Protein Utilizing the AD5, Toxin5, and Allpeptides Databases	Bioinformatic analyses were used to search for sequence similarities between the Cry1A.105 protein and toxins and allergens. The FASTA alignment tool and the allergen (AD5), toxin (TOXIN5), and public domain (ALLPEPTIDES) database sequences were used to assess structural similarity. No significant similarities were found, other than with the Cry1Ac protein; this alignment is not surprising, since the Cry1A.105 protein contains a significant portion of the Cry1Ac protein. The Cry1A.105 protein sequence was also screened against the AD5 sequence database using a pair-wise comparison algorithm. No matches of 8 amino acids or more were found for the Cry1A.105 protein in the AD5 database. No similarities between Cry1A.105 protein and known allergens, human or animal toxins, or pharmacologically active proteins were found in the study. **Classification: ACCEPTABLE**	46694605
In vitro digestibility/ Assessment of the In Vitro Digestibility of the Cry1A.105 Protein in Simulated Gastric Fluid	No bands representative of intact Cry1A.105 protein were identified by SDS-PAGE after ≥30 seconds incubation with simulated gastric fluid containing pepsin. A very faint band of 4.5 kDaltons was observed between the 30 second and 20 minute digestions but was not observed after 20 minutes. The limit of detection for the SDS-PAGE method was determined to be 5 ng for the full-length Cry1A.105 protein. Both the pepsin stability and test material stability controls gave appropriate responses. In the Western Blot assay, Cry1A.105 was not immunologically identifiable within 30 seconds of incubation. The limit of detection for the method was determined to be 1 ng for the full-length Cry1A.105 protein. The pepsin stability and test material stability controls gave appropriate responses. **Classification: ACCEPTABLE**	46694606
Heat stability/ Immunodetection of Cry2Ab2 and Cry1A.105 Proteins in Corn Grain from MON 89034 Following Heat Treatment	The immunodetectability of the Cry1A.105 and Cry2Ab2 proteins in corn grain from MON 89034 following heat treatment was assessed. MON 89034 and conventional grain were ground, mixed with water, and then heated in an oven at 204 ºC for 20 minutes to simulate the heating process used commercially to process grain. Heated and unheated grain was extracted with two buffers: 50 mM CAPS and 50 mM NLS (CAPS containing 2% N-Lauroyl sarcosine). The extracts were analyzed using western blot to detect the presence of the Cry1A.105 and Cry2Ab2 proteins. The amount of immunodetectable Cry2Ab2 protein in either CAPS or NLS buffer extracts of MON 89034 after heating was below the lower LOD. Based on the LOD and the estimated amount of protein in the unheated extract, it was determined that the Cry2Ab2 protein decreased at least 77% and 70%, respectively following heat treatment, relative to their original values. Likewise, the amount of immunodetectable Cry1A.105 protein in either CAPS or NLS buffer extracts of MON 89034 after heating was below the limit of detection (LOD) and had decreased by at least 94% and 78%, respectively, relative to their original values. This loss is likely due to protein degradation or aggregation into an insoluble complex as a result of heat treatment. **Classification: ACCEPTABLE**	46694607
Amino acid sequence comparison/ Bioinformatics Analysis of the Cry1A.105 Protein Utilizing the AD6, Toxin5, and Allpeptides Databases	The amino acid sequence of the Cry1A.105 protein was compared to the sequences of known allergens and toxins using allergen (AD6), toxin (TOXIN5), and public domain (ALLPEPTIDES) databases and the FASTA algorithm. In addition to the FASTA comparisons, the Cry1A.105 protein sequence was compared to the AD6 database using 8 amino acid sliding blocks and the ALLERGENSEARCH algorithm. No proteins were identified with an	46951410

Study Type/Title	Summary	MRID #
	E score of less than 1x10-5 from the search using the AD6 database, indicating that Cry1A.105 has no structural similarity to any known allergens, gliadins, or glutenins. In addition, no matches of 8 or more amino acids were found between the Cry1A.105 protein and sequences in the AD6 database. In the comparisons using the TOXIN5 and ALLPEPTIDES databases, the highest similarity identified was with Bacillus thuringiensis pesticidal protein Cry1Ac (92% identity over an 1,182 aa window and an E score of 0). This result is not surprising, given that the Cry1A.105 protein contains a significant portion of the Cry1Ac protein. No significant similarities between Cry1A.105 protein and known allergens, human or animal toxins, or pharmacologically active proteins were found in this study. **Classification: ACCEPTABLE**	
In vitro digestibility/ Assessment of the in vitro Digestibility of the Cry1A.105 Protein in Simulated Intestinal Fluid	The in vitro digestibility of Cry1A.105 in simulated intestinal fluid containing pancreatin was investigated using western blot analysis. The band for the full-length Cry1A.105 was below the LOD in the 5 minute time-point sample and in the later time-point samples. The LOD for the full-length Cry1A.105 protein by western blot analysis was estimated to be 0.1 ng, which was 0.5 % of the total protein loaded. Therefore, at least 99.5% of the full-length protein was digested within 5 minutes. Bands from proteolytic fragments with approximate molecular weights of 60, 32, and 30 kDa were visible in the five-minute time-point sample. The ~32 kDa fragment was digested and undetectable at the 2-hour time-point and after. The ~30 kDa fragment, which appears as a doublet, was still visible in the 24-hour time-point sample, but its intensity decreased substantially. The ~60 kDa fragment, which also appears as a doublet, represents the trypsin-resistant core and appears to be fairly stable throughout the 24-hour digestion experiment. **Classification: ACCEPTABLE**	46951408

*(Refer, EPA, 2010)

Table 2. Summary of Cry2Ab2 Human Health Data

Study Type/Title	Summary	MRID #
Acute oral toxicity (OPPTS 870.1100)/ Acute Oral Toxicity Study in Mice with Cry2Ab2 Protein	The acute oral toxicity of the Cry2Ab2 protein was assessed in CD-1 mice. Ten male and 10 female mice received E. coli-produced Cry2Ab2 protein at a dose of 2198 mg/kg by oral gavage in two doses (test protein group); ten male and 10 female mice were treated with 2 mM carbonate-bicarbonate, 2mM reduced glutathione buffer (vehicle control group); and ten male and 10 female mice received bovine serum albumin at a dose of 2424 mg/kg (protein control group). Body weight was recorded prior to fasting, prior to dosing, and on days 7 and 14. The test animals were observed for clinical signs of toxicity two times post-dosing and daily for 14 days. A general health/mortality check was done twice daily. All animals were euthanized and necropsied on day 14. All mice survived the study. There were no significant differences in body weight or body weight change among the three groups during the study. The oral LD50 for males, females, and combined mice was greater than 2198 mg/kg. This places Cry2Ab2 protein in TOXICITY CATEGORY III because of dose amounts only; no signs of toxicity were observed. **Classification: ACCEPTABLE**	46951406
In vitro digestibility/Assessment of the in vitro digestibility of the Cry2Ab2 protein in simulated gastric fluid	The in vitro digestibility of Cry2Ab2 in simulated gastric fluid was investigated. No bands representative of intact Cry2Ab2 protein were identified by SDS-PAGE or western blot analysis after ≥ 30 seconds incubation with simulated gastric fluid containing pepsin. In the stained gel, a faint band with molecular weight ~5 kDa was visible in the 30-second time-point, but not in any other samples. No proteolytic fragments were observed in the western blot. The limit of detection (LOD) for the full-length Cry2Ab2 protein by SDS-PAGE with staining was determined to be 5 ng or approximately 0.6% of the total protein loaded. Therefore, at least 99.4% of the full-length Cry2Ab2 protein was digested within 30 seconds. The LOD for the full-length Cry2Ab2 protein by western blot analysis was determined to be 0.2 ng or 1% of the total protein loaded.	46951407

Study Type/Title	Summary	MRID #
	Based on the fact that no band was observed for the Cry2Ab2 protein in the 30 second time-point sample in the western blot and the LOD for the protein using this method, it was concluded that at least 99% of the Cry2Ab2 protein was digested within 30 seconds. **Classification: ACCEPTABLE**	
In vitro digestibility/ Assessment of the in vitro Digestibility of the Cry2Ab2 Protein in Simulated Intestinal Fluid	The in vitro digestibility of Cry2Ab2 in simulated intestinal fluid containing pancreatin was investigated using western blot analysis. The band for the full-length Cry2Ab2 was below the LOD in the 15 minute time-point sample and in the later time-point samples. The LOD for the full-length Cry2Ab2 protein by western blot analysis was estimated to be 0.5 ng, which was 2.5% of the total protein loaded. Therefore, at least 97.5% of the full-length protein was digested within 15 minutes. Bands from proteolytic fragments with approximate molecular weights of 60, 55, 50, 40, 12, and 10 kDa were visible in the five-minute time-point sample. The bands for all of these proteolytic fragments except for the 50 kDa fragment were undetectable at the 24-hour incubation time-point. **Classification: ACCEPTABLE**	46951409
Heat stability/ Immunodetection of Cry2Ab2 and Cry1A.105 Proteins in Corn Grain from MON 89034 Following Heat Treatment	The immunodetectability of the Cry1A.105 and Cry2Ab2 proteins in corn grain from MON 89034 following heat treatment was assessed. MON 89034 and conventional grain were ground, mixed with water, and then heated in an oven at 204 ºC for 20 minutes to simulate the heating process used commercially to process grain. Heated and unheated grain was extracted with two buffers: 50 mM CAPS and 50 mM NLS (CAPS containing 2% N-Lauroyl sarcosine). The extracts were analyzed using western blot to detect the presence of the Cry1A.105 and Cry2Ab2 proteins. The amount of immunodetectable Cry2Ab2 protein in either CAPS or NLS buffer extracts of MON 89034 after heating was below the lower LOD. Based on the LOD and the estimated amount of protein in the unheated extract, it was determined that the Cry2Ab2 protein decreased at least 77% and 70%, respectively following heat treatment, relative to their original values. Likewise, the amount of immunodetectable Cry1A.105 protein in either CAPS or NLS buffer extracts of MON 89034 after heating was below the limit of detection (LOD) and had decreased by at least 94% and 78%, respectively, relative to their original values. This loss is likely due to protein degradation or aggregation into an insoluble complex as a result of heat treatment. **Classification: ACCEPTABLE**	46694607
Amino acid sequence comparison/ Bioinformatic Evaluation of the Cry2Ab2 Protein Utilizing the AD6, TOXIN5, and ALLPEPTIDES Databases	The amino acid sequence of the Cry2Ab2 protein was compared to the sequences of known allergens and toxins using allergen (AD6), toxin (TOXIN5), and public domain (ALLPEPTIDES) databases and the FASTA algorithm. In addition to the FASTA comparisons, the Cry2Ab2 protein sequence was compared to the AD6 database using 8 amino acid sliding blocks and the ALLERGENSEARCH algorithm. No proteins were identified with an E score of less than 1x10-5 from the search using the AD6 database, indicating that Cry2Ab2 has no structural similarity to any known allergens, gliadins, or glutenins. In addition, no matches of 8 amino acids were found between the Cry2Ab2 protein and sequences in the AD6 database. In the comparisons using the TOXIN5 and ALLPEPTIDES databases, the highest similarities identified were with Cry protein homologues derived from Bacillus thuringiensis, Clostridium bifermentans, Paenibacillus popilliae, and Paenibacillus lentimorbus. The results indicate that the Cry2Ab2 protein does not share sequence homology with any proteins that may have adverse effects in humans or animals. **Classification: ACCEPTABLE**	46951411

*(Refer, EPA, 2010)