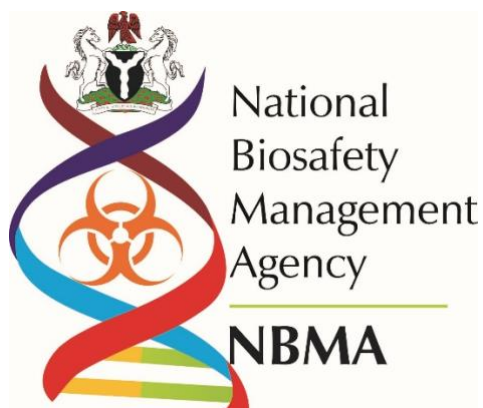




RECOMMENDATION DOCUMENT

RECOMMENDATION OF NATIONAL BIOSAFETY COMMITTEE (NBC) ON AN APPLICATION BY THE INSTITUTE OF AGRICULTURAL RESEARCH (IAR), AHMADU BELLO UNIVERSITY ZARIA AND AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION, FOR GENERAL RELEASE/COMMERCIALISATION OF GENETICALLY MODIFIED COWPEA EVENT 245F CONTAINING *CRY2Ab* GENE CONFERRING RESISTANCE TO MARUCA POD BORER.

APPLICATION REF: NBMA / CM/002

PART A: General Information....

I. Name of Applicants: Institute of Agricultural Research, Ahmadu Bello University, Zaria and, African Agricultural Technology Foundation 4th Floor, Bank of Agriculture Building, 162, Independence Avenue, Central Business District, FCT, Abuja, Nigeria.

II. Contact Details:

- a) **Name of Contact Person:** i. Prof. Mohammed Faguji Ishiyaku
- b) **Postal Address:** Institute of Agricultural Research, Ahmadu Bello University, P.M.B 1044, Zaria, Nigeria.
- c) **Email:** mffaquji@hotmail.com
- d) **Telephone:**(+2348187655504, +2348051316887)
- e) **Fax:** +23469550563

ii. Dr. Tignegre, Jean

Address: AATF Regional Office, 4th Floor, Bank of Agriculture Building, 162, Independence Avenue, Central Business District, FCT, Abuja Nigeria

Telephone: +234 8163964686

E-mail: J.Tignegre@aatf-africa.org

III.

Purpose of application: General Release/Commercialization of Pod Borer-Resistant Cowpea (PBR-Cowpea) Cowpea - *Vigna unguiculata* [L.] Walp.), genetically modified with event AAT-Ø245F-3 (also known as AAT 245F cowpea) containing *Cry2Ab* gene that confers resistance to the pod borer, *Maruca vitrata*.

IV.

Background Information: As of the date of this application, there have been no submissions to other regulatory agencies in other countries for authorization of cowpea with event 245F for general environmental release. However, PBR cowpea with event AAT 709A expressing the Cry1Ab protein for control of the same pest, *Maruca vitrata*, was granted Permit for commercial release in Nigeria and Ghana in 2019 and 2022 respectively.

PART B: Information relating to Genetically Modified Organism (GMO)

I. Description of GMO (including characteristics of donor, recipient, parental organism etc)

Donor Organisms

Bacillus thuringiensis (order: Bacillales; family: Bacillaceae) subspecies *kurstaki* strain HD-1 was the source of the cry1Ab gene used in the development of cowpea with event AAT-Ø245F-3 (245F). *Bacillus thuringiensis* (Bt) is a facultative anaerobic, gram-positive spore-forming bacterium that produces characteristic insecticidal proteins, as parasporal crystals, during the sporulation phase (Sanahuja et al., 2011). These crystals are predominantly comprised of one or more Crystal (Cry) and Cytolytic (Cyt) dendotoxins that are highly specific to their target insect, are innocuous to humans, vertebrates and plants, and are completely biodegradable (Bravo et al., 2007). Over 60 subspecies of *B. thuringiensis* have been described that collectively synthesize various

types of Cry proteins with specific activity against the insect orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, and to nematodes. For instance, the Cyt proteins are mostly found in *B. thuringiensis* strains that are active against Diptera.

Escherichia coli (family Enterobacteriaceae) strain K12, a non-pathogenic strain, was the source of the neomycin phosphotransferase II (NPTII) encoding the *nptII* gene (Beck et al., 1982) and is a gram-negative, motile, facultatively anaerobic rod-shaped bacterium. Certain serotypes are enteropathogenic and are known to cause diarrhoea in infants. Some strains also cause diarrhoea in adults. *Escherichia coli* is a normal inhabitant of the intestinal flora of humans and animals, where it normally does not cause disease.

Characteristics of the recipient organism

Cowpea (*Vigna unguiculata* ssp. *unguiculata* L Walp.) is a drought tolerant, heat adapted herbaceous legume. It is also known as black-eyed pea, niebe, Southern pea among others. It thrives in the Sahel and the dry moist Guinea savannahs. Cowpea is productive in places with erratic rainfall and soils that are poor in organic matter and soil nutrients. African cowpeas can have a predominantly bushy upright habit or take the form of a prostrate crawling plant serving as an excellent ground cover to preserve soil moisture with its unique ability to fix soil nitrogen. Cowpea breeders have developed high yielding cowpea varieties producing large white seed with rough seed coats, the preferred type for consumption and regional trade. The yield potential is in the range of 1500-2000kg/ha but these yields are rarely achieved in farmer's fields in SSA. Depending on the location and year, typical yields range from 150-400 kg/ha. One of the main reasons for the huge yield gap is the biological constraints, particularly insect pests that affect this otherwise hardy crop.

I. Characteristics of vector (nature, source etc)

Nature and source of the vector: The transformation vector used was plasmid pMB6 (Figures 2 and 3) which contains two expression cassettes within the T-DNA (summarized in Table 4). The *Agrobacterium tumefaciens*-mediated transformation of organogenic seed explants with plasmid pMB6 resulted in the introduction of the *cry2Ab* gene from *B. thuringiensis* subsp. *kurstaki* strain HD-1 and the neomycin phosphotransferase II encoding gene (*nptII*) from the Tn5 transposon of *Escherichia coli* strain K12 as a selectable marker. Molecular characterization of the introduced DNA

within 245F cowpea confirmed the presence at a single insertion site of the transfer-DNA (T-DNA) region derived from plasmid pMB6 that was stably inherited over multiple generations as a single genetic locus according to Mendelian rules of inheritance. Nucleotide sequencing of the entire inserted DNA, including portions of the 5' and 3' flanking cowpea genomic sequence, was performed to confirm its organization and the potential for creating any new open reading frames. The inserted DNA was comprised of one complete copy of the plasmid pMB6 T-DNA, except for 41-bp and 26-bp truncations at the 5' and 3' termini, respectively. From the analysis of 5' and 3' flanking host genomic DNA, the site of T-DNA insertion within 245F cowpea was mapped to an inter-genic region on chromosome 1, and there were no new novel junction-spanning open reading frames created as a consequence of the genetic transformation.

I. Genetic modification of organism(s)

The Agrobacterium-mediated transformation of embryonic cells event stack contains the *cry2Ab* gene isolated from *Bacillus thuringiensis* and the *nptII* gene from a non-pathogenic strain of *Escherichia coli* (family *enterobacteriaceae*) strain K12.

✓. Nature of the final GMO (Description of genetic trait or phenotypic characteristics, etc)

Description of genetic trait or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed

Pod Borer-Resistant Cowpea (PBR-Cowpea) Cowpea - *Vigna unguiculata* (L.) Walp., genetically modified with events; event AAT-Ø245F-3 (also known as AAT 245F cowpea) containing the gene Cry 2Ab that confers resistance to the pod borer, *Maruca vitrata*. The event is morphologically similar to the conventional counterpart except for this new trait.

PART C: Information relating to the condition for release and the receiving environment

I. Information on the Release (Description of the proposed deliberate release, etc)

Upon receipt of Biosafety Approval, crosses will be developed to combine cowpea varieties containing event AAT245F and AAT709A. The general release of the combined events is therefore not anticipated immediately but can be expected in two to three seasons. The resultant breeding stack will be released in major cowpea growing areas across Nigeria, to assess their performance across different environments.

Preparation of the site prior to the release

Since cowpea with event AAT 245F does not differ from traditional cowpea except for resistance to pod borer trait, site preparation is anticipated to be the same as for conventional cowpea.

PART D: Information relating to the interactions between the genetically modified organisms or products and the environment

- I.** Characteristics and Factors affecting survival Multiplication, Gene Expression and Dissemination (Biological features, Known or predicted environmental conditions, etc.)

The characteristics and factors affecting survival, multiplication, gene expression and dissemination are not different from those of the unmodified cowpea counterparts.

- II.** Interactions with the Environment (Predicted habitat of the GMO, Studies of the behaviour and characteristics etc)

Interactions with the environment are not different from those of the conventional counterpart, except in the insect resistance trait for which the product was modified.

PART E: Information on monitoring control, wastes treatment and emergency response plans

I. Monitoring Techniques
<p>The presence of event AAT245F in cowpea products can be identified by employing different techniques. Nucleic acid-based methods such as Southern blot or PCR techniques can identify the inserted nucleotide sequences, while specific ELISAs can detect the presence of the, NptII and Cry2Ab proteins in individual plants or in specific tissues. Emergency response will be in accordance with NBMA's Guidelines.</p>

II. Control of the Release (Methods and procedures, etc)
<p>Application to introduce cowpea with event AAT245F, if approved will lead to availability of seed to farmers in the territory of Nigeria where pod borers are present. After approval, the crop will be used in all places where conventional cowpea is used. Escape may therefore apply only during confined field trials but not in the release scenario.</p>
III. Wastes Treatment (Type, expected amount, etc)
<p>There are no biological wastes associated with cultivation of cowpea with event AAT245F or any other cowpea for that matter. Cowpea is mainly cultivated for grain, and the vegetable which also offers fodder to livestock.</p>
IV. Emergency Response Plan (Methods and procedures for control, etc)
<p>In the event of a need for emergency recall, the NBMA Guidelines for Emergency response should be applied.</p>

PART F: Risk Assessment Parameters

I. Characteristics of the Organism

Donor Organism: The safety assessment of Cry2Ab and NPTII was conducted following a “weight-of-evidence” approach that considered amino acid sequence similarity to known toxins or allergens, digestibility under standardized *in vitro* conditions, and appropriate oral toxicity studies. Bio-informatics analyses of both Cry2Ab and NPTII found no significant sequence similarities with known mammalian toxins and allergens. The Cry2Ab and NPTII proteins were rapidly degraded in simulated gastric fluid containing pepsin and neither protein was acutely toxic in mice when tested at dosages up to 2,198 mg/kg body weight and 5,000 mg/kg body weight, respectively. Relative to the highest estimates of potential human dietary exposure, the concentrations of Cry2Ab and NPTII proteins used to demonstrate lack of acute oral toxicity represented safety margins of 603-fold and more than one million-fold, respectively. There are no conceivable exposure scenarios, whether for infants consuming weaning foods containing 245F cowpea or any other population sub-group consuming products derived from 245F cowpea, that would result in less than acceptable safety margins for Cry2Ab and NPTII.

The experience with genetically engineered crop plants with introduced traits conferring insect-resistance and/or herbicide-tolerance has indicated that the incorporation of these traits have not had a biologically meaningful impact on the composition of key nutrients and anti-nutrients. As part of the weight-of-evidence approach for evaluating whether there were any unanticipated consequences of the genetic modification, compositional analyses were performed to compare concentrations of key nutrients and anti-nutrients in samples of cowpea grain and leaves collected from transgenic event 245F and non-transgenic control cowpea grown during the 2020 season at six different locations representing typical cowpea growing conditions in West Africa. The compositional assessment of 245F cowpea included analyses for proximates, fibre, and calcium in samples of leaves, and analyses for proximates, fibre, minerals, amino acids, vitamins, and key anti-nutrients in grain samples.

Recipient Organism: Cultivated cowpeas have a high level of self-pollination. Based on the work of Blackhurst and Miller (1980) in Texas. It is noted that the pollination process in cultivated cowpeas is complete before the flower opens. Cowpea has a long history of safe consumption by humans and animals and the modification does not alter its biology. Other cowpea products are also used as food, such as green leaves, immature snapped pods, and green cowpea seeds.

II. Characteristics of the vector(s)

Nature and source of the vector: Transgenic cowpea event AAT-Ø245F-3 (hereafter 245F cowpea) was produced by *Agrobacterium tumefaciens*-mediated transformation of organogenic seed explants with plasmid pMB6 resulting in the introduction of the *cry2Ab* gene from *B. thuringiensis* subsp. *kurstaki* strain HD-1 and the neomycin phosphotransferase II encoding gene (*nptII*) from the Tn5 transposon of *Escherichia coli* strain K12 as a selectable marker.

III. Characteristics of Genetically Modified Organisms

Transgenic cowpea with event AAT-Ø245F-3 expresses *Cry2Ab* protein which confers protection against Lepidopteran insects as *Maruca vitrata*. The event is morphologically same as it's conventional counterparts except for the new trait. It was produced by *Agrobacterium tumefaciens*-mediated transformation of organogenic seed explants with plasmid pMB6 resulting in the introduction of the *Cry 2Ab* gene from *B. thuringiensis* subsp. *kurstaki* strain HD-1 and the neomycin phosphotransferase II encoding gene (*nptII*) from the Tn5 transposon of *Escherichia coli* strain K12 as a selectable marker.

IV. Characteristics of Resuscitated Organisms and Genes and Fossils DNA sequences, Resuscitated Organisms
Not applicable
V. DNA sequences from Fossils or from Resuscitated Organisms
Not applicable
VI. Safety Consideration for Human and Animal Health

History of safe use: as detailed in the application dossier, the expressed proteins Cry2Ab and neomycin phosphotransferase II (NPTII) have a history of safe use in approved genetically engineered crops in many countries worldwide.

Potential toxicity:

Under several conditions of testing as outlined in the application dossier, oral administration of NPTII test substance at concentrations up to 5,000 mg/kg body weight produced no test substance-related clinical signs of toxicity, body weight losses, gross lesions, or mortality, further substantiating the predicted lack of acute oral toxicity of NPTII.

Also for *Cry2Ab*, under conditions of different tests as contained in the dossier, the acute oral dose resulting in 50% lethality (LD₅₀) of the Cry2Ab protein in mice was greater than 2,198 mg/kg body weight. This dose was determined to be of no observed effect level (NOEL) in the study. In summary, using conservative search criteria, it was concluded that the *Cry2Ab* query sequence showed no significant sequence similarity to any proteins known, or suspected, to be of mammalian toxicological concern. This conclusion is further supported by the numerous reviews of similar *Cry2Ab* bioinformatic analyses by regulatory authorities who have determined that Cry2Ab does not share structural similarities with proteins toxic to humans or livestock animals (CERA, 2013).

Potential Allergenicity:

As part of assessing the potential allergenicity of the Cry2Ab protein, an evaluation of the significance of any amino acid sequence similarity with known allergens was conducted (MacKenzie, 2020). Based on the results of the assessment, the lack of potentially significant sequence similarity between the Cry2Ab protein and known and putative allergens indicates that it is not a known allergen and is unlikely to be cross-reactive to known allergens.

For NPTII, protein evaluations of the significance of any amino acid sequence similarity with known allergens were conducted as detailed in the application dossier.

Based on the results of these analyses, the lack of potentially significant sequence similarity between the NPTII protein and known and putative allergens indicated that it is not a known allergen and is unlikely to be cross-reactive to known allergens.

Substantial equivalence:

From the report of the compositional analysis outlined in the dossier, no consistent patterns emerged to suggest that biologically meaningful changes in composition or nutritive value of the leaves or grain of the cowpea under reviews had occurred as an

unintended consequence of the genetic modification. Thus, leaves and grains derived from 245F cowpea are compositionally equivalent to their conventional counterparts.

Other nutritional details:

Reported studies show that there were no increases in anti-nutritional factors in cowpea, such as phytate and oligosaccharides.

VII. Environmental considerations

Non target organisms:

No adverse impact of cowpea with event AAT 245F on the abiotic environment is expected to result from the general release of this cowpea into the Nigerian environment. In summary, studies on effect on biodiversity showed no effects on non-target organisms.

Invasiveness and weediness:

Field comparisons between 245F cowpea and its near-isogenic non-transgenic counterpart over multiple locations and years did not identify any significant differences in plant growth and morphology, reproductive biology characteristics, seed set or shattering, that would indicate any unintended change in the weediness or invasiveness of conventional cowpea cultivars (Section 8) (Abdourhamane et al., 2021a).

Horizontal gene flow:

Pollen-mediated gene flow is a natural part of the reproductive biology of all plants and is important in the maintenance of genetic variation in populations, as well as in the spread of new traits among populations and across species boundaries (Tzotzos et al., 2009). Pollen movement between plants is dependent upon wind or animal vectors (pollinators), and while it occurs with all crop species, the amount of gene flow (i.e., out-crossing) is a function of crop biology. Gene flow between cultivated plants and their wild relatives occurs naturally for all crops with relatives that are sexually compatible and grow in proximity to the crop (Ellstrand et al., 1999; Raybould, 2010b; Raybould and Wilkinson, 2005). Therefore, studies on possible gene flow to wild cowpea carried out, showed no evidence of horizontal gene flow.

III. Socio-economic consideration

Introduction of cowpea with event AAT 245F is not expected to alter any of the existing socio and economic patterns in agricultural systems in Nigeria as its agronomic

practices will be same as those of conventional cowpea varieties grown in Nigeria. However, farmers will save on the resources used in spraying for the target pests as well as gain income from the saved yield losses. An annex of socio-economic perspectives is provided in the Application.

PART G: Information on post release monitoring and emergency response plans

Cowpea with event AAT 245F has no adverse environmental impact and should any seed be dispersed; this could only lead to the appearance of volunteers. Cowpea volunteers are however easily controlled through normal agricultural practices. In addition, cowpea cannot survive without human intervention and the probability of any volunteers, resulting from seed dispersal, becoming weeds and being persistent in the environment is therefore negligible. In addition, post release monitoring and emergency responses will also follow NBMA's Guidelines.

Terms and Conditions

Recommendation:

National Biosafety Committee, hereby recommends the approval of this application, by the National Biosafety Management Agency.

NBC Members

Name	Organization	Signature
Dr. Muhammed Ishaq	NCRI, Baddegi	
Mr. S.G. Sulola	Nigeria Custom Service	
Mr. Benson O. Ajibi	NAQS	
Dr. Nkechi Mba	FCCPC	
Mrs. Haleemat Kadiri	NAFDAC	

