



RECOMMENDATION DOCUMENT

**RECOMMENDATION OF NATIONAL BIOSAFETY COMMITTEE (NBC) ON AN APPLICATION BY INSTITUTE OF AGRICULTURAL RESEARCH (IAR), AHMADU BELLO UNIVERSITY ZARIA FOR GENERAL RELEASE/COMMERCIALISATION OF COWPEA GENETICALLY MODIFIED FOR INSECT RESISTANCE (AAT 709A)
APPLICATION REF: NBMA / CM/002**

PART A: General Information....

I Name of Applicant: Institute of Agricultural Research, ABU, Zaria

II Contact Details:

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III. Purpose of application - General release of Pod Borer-Resistant Cowpea (PBR-Cowpea) (*Vigna unguiculata* (L.) Walp.) Event AAT 709A (OECD unique identifier AAT-709AA-4) Expression of the Cry1Ab protein that confers protection from certain lepidopteran insect pests of cowpea, principally the pod

borer (<i>Maruca vitrata</i> Fabricius [Lepidoptera:Crambidae]).
IV. Background Information Applications for CFT of this product were approved in 2009 and in 2018. This is now an application for general release of the same product. Thus, it has been a ten year study, with single and multilocational trials, before this application.

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-709AA-4 (709A). *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 Cytotoxic (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 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

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 pMB4 (Figures 5 and 6) which was derived from the binary vector pART27 (Gleave et al. 1992; GenBank accession X69707.1), the backbone of which carries the RK2 minimal replicon for maintenance in *Agrobacterium*, the ColE1 origin of replication for high-copy maintenance in *Escherichia coli*, and the Tn7 spectinomycin/streptomycin resistance gene as a bacterial selectable marker (*aadA*). The DNA region between the left and right T-DNA borders of the transformation plasmid includes expression cassettes for *cry1Ab* and the selectable marker gene, neomycin phosphotransferase II (*nptII*).

I. Genetic modification of organism(s)

The *Agrobacterium*-mediated transformation of the cowpea cultivar IT86D-1010 using plasmid pMB4 was essentially as described by Popelka et al. (2006) and modified by Higgins et al. (2012). The details are given.

IV. 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; PBR Cowpea event AAT709A expresses *Cry1Ab* protein which confers protection against Lepidopteran insect pests such as *Maruca vitrata*. PBR Cowpea event AAT709A 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)

The proposed dates for release are 2019/2020. This will be followed by variety evaluation, official release and seed production. AAT 709A seed is expected to be produced and remain on the market for approximately 5 years. After that time, it will be replaced by a stack event product that expresses both Cry1Ab and Cry2Ab proteins, which is expected to increase the durability of resistance to Maruca spp.

Preparation of the site prior to the release

Since cowpea with event AAT 709A does not differ from traditional cowpea except for resistance to pod borer, site preparation is anticipated to be the same as for traditional cowpea.

The current application seeks for consideration and general release approval to permit cultivation and placing on the market of Cowpea with event AAT 709A in Nigeria

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 counterpart.

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	
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<p>The presence of event AAT 709A in cowpea or cowpea products can be determined by employing different techniques. Nucleic acid-based methods such as Southern blot or PCR techniques, sequencing techniques, can identify the inserted nucleotide sequences. Emergency response will be according to NBMA guidelines.</p>	
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II. Control of the Release (Methods and procedures, etc)	
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<p>It is a general release</p>	
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III. Wastes Treatment (Type, expected amount, etc)	
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<p>Not applicable</p>	
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IV. Emergency Response Plan (Methods and procedures for controlling, etc)	
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<p>In the event of a need for emergency recall, the NBMA guidelines for emergency response should be applied.</p>	
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PART F: Risk Assessment Parameters

I. Characteristics of the Organism

Donor Organisms. (a)(i) *Bacillus thuringiensis* (order: Bacillales; family: Bacillaceae) subspecies *kurstaki* strain HD-1 was the source of the cry1Ab gene used in the development of event AAT-7Ø9AA4 (709A) cowpea. *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) δ -endotoxins 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 also to nematodes. The Cyt proteins are mostly found in *B. thuringiensis* strains that are active against Diptera.

(ii) *Escherichia coli* *Escherichia coli* (family Enterobacteriaceae) strain K12, a non-pathogenic strain, was the source of the neomycin phosphotransferase II (NPTII) encoding *nptII* gene (Beck et al., 1982) and is a gram-negative, motile, facultative 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 generally does not cause disease. *E. coli* has been developed as a model organism for the study of microbiology, biochemistry and molecular biology and is one of the most thoroughly characterized of organisms. Laboratory strains such as *E. coli* K12 have become "attenuated" through multiple years of propagation in the laboratory environment and have largely lost the ability to colonize the intestine. (EPA 1997).

Characteristics of the recipient organism - cowpea. The biology and ecology of cowpea is described in detail in the OECD publication ENV/JM/MONO(2015)48 "Consensus document on the biology of cowpea (*Vigna unguiculata* (L.)Walp.). Excerpts are presented below: 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 that affect this otherwise hardy crop

II. Characteristics of the vector(s)

Nature and source of the vector: The transformation vector used was plasmid pMB4 which was derived from the binary vector pART27 (Gleave et al. 1992; GenBank accession X69707.1), the backbone of which carries the RK2 minimal replicon for maintenance in *Agrobacterium*, the ColE1 origin of replication for high-copy maintenance in *Escherichia coli*, and the Tn7 spectinomycin/streptomycin resistance gene as a bacterial selectable marker (*aadA*). The DNA region between the left and right T-DNA borders of the transformation plasmid includes expression cassettes for *cry1Ab* and the selectable marker gene, neomycin phosphotransferase II (*nptII*).

III. Characteristics of Genetically Modified Organisms	
<p>PBR Cowpea event AAT709A expresses Cry1Ab protein which confers protection against Lepidopteran insect pests such as <i>Maruca vitrata</i>. PBR Cowpea event AAT709A is morphologically similar to the conventional counterpart except for this new trait. Agrobacterium-mediated transformation of the cowpea cultivar IT86D-1010 was carried out using plasmid pMB4 as described by Popelka et al. (2006) and modified by Higgins et al. (2012).</p>	
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	
<p>History of safe use: Sufficient evidence of history of safe use of the Cry1Ab and <i>neomycin</i></p>	

phosphotransferase II (nptII) genes, the donor organism *Bacillus Thuringiensis*, and the recipient *Vigna Unguiculata* has been provided in the dossier.

Potential Toxicity:

Data from similarity searches with the amino acid sequences show no similarity between the Cry1Ab protein and E.Coli nptII to known toxins.

Pepsin digestion stability studies were reported and the cry 1AB protein showed no stability to the pepsin. No heat stability studies were presented.

Potential Allergenicity:

Data from similarity searches with the amino acid sequences show no relevant similarity between the Cry1Ab protein and E. Coli nptII to known allergens.

Substantial Equivalence:

Compositional analysis of the gm cowpea grain leaves and folder showed substantial equivalence with the conventional counterpart.

Other Nutritional Details:

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

VII. Environmental considerations	
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Non-Target Organisms:	
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Studies on effect on biodiversity showed no effects on non-target organisms.	
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Invasiveness and Weediness:	
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Results of studies provided show no tendency to weediness or invasiveness.	
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Horizontal Gene Flow:	
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Studies on possible gene flow to wild cowpea carried out showed no evidence of horizontal gene flow.	
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III. Socio-economic consideration	
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The introduction of the Bt Cowpea will not stop the continued use of unmodified farmer preferred varieties by any farmer who chooses to do so. The use of the Bt cowpea will increase farmers' wealth from increased yield and reduce Farmer investment in pesticides, it will reduce environmental pollution by the insecticides due to reduced amount of total insecticide sprayed, it will reduce farmers' health challenges from insecticide exposure.	
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Introduction of Bt Cowpea will translate to improved food security in the entire country due to availability of much higher amounts of cowpea. This will also translate to higher incomes due to export of the commodity, because less residual insecticide means higher acceptability of Nigerian cowpea in the international market.	
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PART G: Information on post release monitoring and emergency response plans

Post release monitoring and emergency response should follow NBMA guidelines.

Terms and Conditions	
<ol style="list-style-type: none">1. All the study reports submitted should be incorporated appropriately into the dossier.2. All editorial errors should be amended as listed in the Deficiency notes provided, including –<ol style="list-style-type: none">a. Pg 62 - Scientific name and Taxonomy: Vigna Ungiculata instead of <i>Vigna unguiculata</i>b. Some references in the text do not appear in the list of references eg. Ige et al 2011 on pg 65; Morse et al pg 83, AATF 2017 pg 103c. Referencing style should be consistent eg alphabetical order is not maintained in some instances.d. In a number of instances, the event name AAT 709A was written just as 709A.e. There was an instance of T-DNA being written as TDNA, eg. Pg 75.f. nptII is sometimes written as NPTII.g. If there are more than two contact persons, their details should come in consistent order, ie, the details of the first mentioned person should continue to come first.	
Recommendations	
<p>Subject to the above terms and conditions and deficiency notes, the National Biosafety Committee, hereby recommends the approval of this application by the National Biosafety Management Agency.</p>	

Names, Signatures and Date Of NBTS Members	