National Biosafety Authority

DECISION DOCUMENT FOR ENVIRONMENTAL RELEASE OF GENETICALLY MODIFIED PLANT

Tracking No: 2023-030-SARI-002-D **Date: 31st October 2023**

Title: Decision on an Application from the Council for Scientific and Industrial Research - Savanna Agricultural Research Institute (CSIR-SARI) for the Environmental Release of Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to *Maruca* Pod Borer (*Maruca vitrata*) Event AAT-Ø245F-3 (hereafter referred to as Cowpea Event 245F) in Ghana.

Legislation

Pursuant to Sections 4, 12, 17, 18, 19, 21, 22 and 23 of the Biosafety Act, 2011 (Act 831) and the relevant procedures under the Biosafety (Management of Biotechnology) Regulations, 2019 (L.I. 2383), the Board of the National Biosafety Authority (NBA) evaluated information submitted by the applicant - the Council for Scientific and Industrial Research - Savanna Agricultural Research Institute (CSIR-SARI). This information addressed the safety of the insect resistance Cowpea Event 245F. The Board of the NBA has determined that the genetically modified plant does not present an altered environmental risk or a food or feed safety concern when compared to conventional cowpea varieties in Ghana. **The Board has therefore approved the environmental release of Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to *Maruca* pod borer (*Maruca vitrata*) Event AAT-Ø245F-3 for three (3) year period, renewable administratively.**

1.0 Short Summary of the Genetically Modified Organism (GMO)

Id. of the Modified Plant:	Cowpea Event 245F, OECD Unique Identifier **AAT-Ø245F-3**
Applicant:	Council for Scientific and Industrial Research, Savanna Agricultural Research Institute (CSIR-SARI)
Plant Species:	Cowpea. *Vigna unguiculata* L. Walp.
Modified Traits:	Insect pest resistance *(Maruca vitrata)*
Trait Introduction Method:	*Agrobacterium tumefaciens*-mediated transformation
Intended Use(s) of the Modified Plant:	CSIR-SARI intends to release into the environment, cowpea event 245F (with minimal restrictions related to reproductive isolation and physical or biological confinement, including material management). The environmental release would allow for Event 245F to be used as a breeding parent together with the previously authorized Event 709A (AAT-7Ø9AA-4) to create a stacked-event with both the CrylAb and Cry2Ab insecticidal proteins offering protection against *Maruca vitrata.* This would ultimately help implement the insect pest *(Maruca vitrata)* resistance management plan.

2.0 Application Summary

The applicant noted that cowpea (*Vigna unguiculata* L. Walp.) is an important staple in the diets of more than 200 million households as well as serving as fodder in sub-Saharan Africa (SSA). However, production of the crop is reduced with yield losses ranging from 20–80 percent as a result of insect pests. The primary pest is *Maruca* pod borer *(Maruca vitrata).*

Cowpea pod borer has been controlled primarily by application of synthetic insecticides, although in some cases resistance to these has developed. Additionally, cost of insecticides and the cost of labour used in applying the insecticides are often out of the reach of growers. Insecticide use also poses health risks to growers and their families owing to a lack of adequate training and the limited use of personal protective equipment. Low literacy rates also affect the ability of many farmers to read and adhere to application instructions. Thus, any intervention that can reduce dependency on insecticide use is a welcome development. To provide an additional tool for the control of *M. vitrata,* cowpea was bioengineered for resistance to lepidopteran insect pests through the production of the Cry2Ab insecticidal protein from the widely used biopesticide derived from *Bacillus thuringiensis* (Bt).

Cowpea was genetically modified to express the trait of resistance to the legume pod borer, *M. vitrata*, (Lepidoptera: *Crambidae*). Genetically modified 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. The Cry2Ab and neomycin phosphotransferase II (NPTII) proteins have a history of safe use in approved genetically modified crops in many countries worldwide.

The applicant provided data on the identity of Cowpea Event 245F, a detailed description of the transformation method, data and information on the insertion site, gene copy number and levels of gene expression in the plant and the role of the inserted genes and regulatory sequences. The newly produced proteins (Cry2Ab and NPTII) were identified and characterized.

The pod borer resistant 245F cowpea will not be placed on the market (commercialized) as a standalone product but will be stacked with other cowpea varieties containing event 709A using conventional breeding. The new varieties which shall be submitted for variety registration will produce both the Cry1Ab protein, derived from event 709A, and the Cry2Ab protein, derived from 245F cowpea. It is expected that these new cowpea varieties carrying more than one protective gene would deliver increased protection against the damage caused by *M. vitrata.*

The genetic modification resulting in Cowpea Event 245F was not intended to affect a specific agronomic or phenotypic characteristic, except to confer resistance to lepidopteran pests, such as the cowpea pod borer. To confirm the lack of any unexpected, unintended, consequences from the genetic modification resulting in Cowpea Event 245F, agronomic performance and other phenotypic measurements were collected from field-grown plants at six locations in West Africa in 2020.

The agronomic and phenotypic data generated for event 245F, and the control IT86D-1010 cowpea support the conclusion that the genetic modification resulting in Event 245F did not have an unintended effect on normal plant growth and development. Observations of significant reductions in both pod and grain damage due to *M. vitrata* were consistent with the intended effect of the introduced *cry2Ab* gene. From the data and observations, there were no indications that Event 245F cowpea would be more invasive or persistent in the environment than conventional cowpea.

The data presented in this submission have not identified potential environmental hazards or health and safety concerns relative to the conventional cowpea. In addition, food and feed derived from 245F cowpea are as safe as those derived from conventional cowpea varieties.

Furthermore, the applicant has provided the NBA with an event-specific method for the detection and identification of Cowpea Event 245F to aid surveillance.

3.0 Risk Assessment

3.1 Criteria

The Board of the NBA reviewed the risk assessment report from the Technical Advisory Committee (TAC) on the application in accordance with the criteria for assessing environmental safety of GMOs and assessing food and feed safety of GMOs as provided in the legislation. The risk assessment review considered the potential of the:

- weediness and or invasiveness of Cowpea Event 245F;
- pollen mediated gene flow from Cowpea Event 245F to wild relatives whose hybrid offspring may become weedier or more invasive;
- Cowpea Event 245F to become a plant pest;
- impact of Cowpea Event 245F and its gene products on non-target species, including humans;
- impact of Cowpea Event 245F on biodiversity;
- impact of Cowpea Event 245F on human nutrition and health;
- impact of Cowpea Event 245F on livestock nutrition and health;
- impact of Cowpea Event 245F on workers and processors; and
- development of resistant *Maruca* population;

3.2 Development Method

Cowpea Event 245F was developed through *Agrobacterium tumefaciens*-mediated transformation of cowpea cultivar IT86D-1010 using plasmid pMB6, which contains two gene expression cassettes within the T-DNA. This resulted in the introduction of the *cry2Ab* gene from *Bacillus 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.

Based on the information provided by the applicant, and experience with the Cry2Ab and neomycin phosphotransferase II (NPTII) proteins, having a history of safe use in approved genetically modified crops in many countries, the Board of the NBA concludes that there are no safety concerns with respect to the development process.

3.3 New Traits

3.3.1 Insect Resistance

Cowpea Event 245F was developed to express a new trait of resistance to the cowpea pod borer, *Maruca vitrata*. No existing endogenous traits of the parent species were intentionally altered. Except for the intended resistance to Maruca pod borer, 245F cowpea is agronomically, phenotypically, and compositionally equivalent to the conventional cowpea.

The first cassette contains the *cry2Ab* encoding gene from *Bacillus thuringiensis* subs. *kurstaki* strain HD-1. The nucleotide sequence of the *cry2Ab* gene was codon-optimized for plant

expression and includes one extra amino acid near the N-terminus, aspartic acid, which was introduced during cloning. The *cry2Ab* coding sequence encodes a 634-amino acid protein (70.9 kDa) corresponding to the full-length protein (GenBank accession no. AAA22342.1). The N-terminus of the Cry2Ab coding sequence was fused in-frame with the 273-bp *Arabidopsis thaliana* SSU leader and chloroplast transit peptide (CTP) (GenBank accession no. X68342.1). The *cry2Ab* gene is under the control of the promoter and 5' UTR of the RUBISCO SSU from *A. thaliana* (GenBank accession no. X13611.1) with transcription termination sequences derived from the tobacco (*Nicotiana tabacum*) RUBISCO SSU. Expression of *cry2Ab* confers resistance to the crop against the lepidopteran pest (*M. vitrata)*, also known as cowpea pod borer.

The second cassette contains a copy of the *nptII* encoding gene from the Tn5 transposon of *Escherichia coli* strain K12. To eliminate leaky expression of *nptII* in Agrobacterium and prevent selection of false-positive kanamycin-resistant plants, the *nptII* gene was interrupted with a modified catalase-1 (CAT-1) intron from castor bean (*Riccinus communis*). Transcription of the *nptII* gene is controlled by the S1 promoter from *Subterranean clover stunt virus* (SCSV) and termination sequences are provided by the SCSV DNA segment 3. The *nptII* gene functions as a selectable marker immediately following transformation of plant cells during *in vitro* tissue culture.

In order to estimate potential human and livestock exposure to the Cry2Ab and NPTII proteins produced in 245F cowpea, the concentration of these proteins in plant tissues representing likely exposure pathways (i.e., seeds and leaves) was determined by quantitative enzyme-linked immunosorbent assay (ELISA). Samples of leaves and dry seed were collected from 245F, and control IT86D-1010 plants grown under confined field trial conditions at three locations in Nigeria and three locations in Ghana in 2020. These same locations were used to produce material for composition analysis and for the collection of agronomic and phenotypic data.

The N-terminus of the Cry2Ab coding sequence is fused in-frame with the 273-bp *Arabidopsis thaliana* SSU leader and CTP (GenBank accession no. X68342.1), which results in localization of the Cry2Ab protein in the green tissues of event 245F plants. Concentrations of Cry2Ab in samples of leaf tissue ranged from 34.6–442.5 ng/mg tissue, with an average concentration of 192.9 ng/mg tissue. As a result of the chloroplast directed accumulation of Cry2Ab protein, the concentration in 245F cowpea seed samples was below the limit of quantification (LOQ) of 2.4 ng/g tissue.

Expression of the *nptII* gene is controlled by the constitutive S1 promoter from SCSV and accumulation of some amount of NPTII protein would be expected in all plant tissues. Across locations, concentrations of NPTII protein ranged between 0.08–0.26 ng/mg tissue and 2.2–3.3 ng/mg tissue in leaf and seed tissue samples, respectively. No quantifiable amounts of Cry2Ab and NPTII proteins were found in any tissue samples derived from the control (conventional cowpea).

The patterns of expression of the *cry2Ab* and *nptII* genes were consistent with the known specificity of the respective transcriptional promoters and, in the case of Cry2Ab, the post-translational targeting to the chloroplast.

The assessment of potential dietary exposure to new or novel proteins in the diet is an essential component of food and feed safety assessment. Considering the food uses of cowpea, likely routes of dietary exposure to Cry2Ab and NPTII would be via consumption of leaves, pods, and grain (dry seed). As estimates of consumption were not available for pods, the exposure assessment focused on grain (seed) and leaf consumption. For Cry2Ab, the value used for seed was the LOQ of 0.0024 µg/g, while the value used for leaves was the highest measured value of 442.5 µg/g. The highest measured NPTII concentrations of 3.3 µg/g and 0.26 µg/g were used for seed and leaves, respectively.

Conservative estimates of dietary exposure to Cry2Ab and NPTII proteins for livestock animals consuming forage, hay, and seed derived from 245F cowpea were also developed. The highest

exposure to Cry2Ab protein in 245F cowpea would be for lambs consuming a diet containing up to 100 percent fodder, where daily dietary intake for Cry2Ab was estimated at ca. 61.5 mg/kg body weight. For all livestock species, exposure to Cry2Ab protein from cowpea seed was estimated as negligible, ranging from 0.01–0.09 µg/kg body weight. The highest exposure to NPTII would be for lambs consuming a diet containing 75 percent seed, where daily intake was estimated at ca. 0.12 mg/kg body weight. It is unlikely that all of the cowpea fodder or seed in a feed ration would be substituted with 245F cowpea. Hence, realistic dietary exposures are likely to be less.

The applicant provided information on weight-of-evidence supporting the lack of identifiable hazards associated with Cry2Ab protein as below:

- The Cry2Ab binds to specific receptors on the brush border membrane of midgut epithelial cells found only in target lepidopteran insect species initiating subsequent reactions that result in cell lysis and septicemia causing insect death. This does not occur in mammals, other vertebrates, or other insects because they lack the specific receptors for Cry2Ab.
- Bioinformatic analyses showed that the Cry2Ab protein does not display significant amino acid sequence similarity to known or putative protein mammalian toxins or allergens.
- The Cry2Ab protein is rapidly degraded in SGF containing pepsin, with more than 99 percent digestion within 30 seconds as assessed by SDS-PAGE and Western Immunoblot analysis.
- The Cry2Ab protein is not acutely toxic in mice at dosages up to 2,198 mg/kg body weight, the highest dosage tested.

The applicant also provided information on weight-of-evidence supporting the lack of identifiable hazards associated with NPTII protein as below:

- The known function of NPTII in catalyzing the ATP-dependent phosphorylation of the 3'-hydroxyl group of the amino-hexose portion of certain aminoglycosides is not similar to the activities of known protein toxins.
- Bioinformatic analyses showed that the NPTII protein does not display significant amino acid sequence similarity to known or putative protein toxins or allergens.
- The NPTII protein is rapidly degraded in SGF containing pepsin and SIF containing pancreatin. No intact NPTII or NPTII-derived fragments were detected after exposure to pepsin-containing SGF, or pancreatin-containing SIF as assessed by Western Immunoblot labelling following SDS-PAGE analysis.
- The NPTII protein is not acutely toxic in mice at dosages up to 5,000 mg/kg body weight, the highest dosage tested.

Based on the available evidence, the Board of the NBA concludes that the Cry2Ab and NPTII proteins produced in Cowpea Event 245F are unlikely to be toxic or allergenic to mammals.

3.3.2 Antibiotic Resistance Marker Genes

Regarding the presence of the *nptII* gene in Cowpea Event 245F the probability that this gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract is considered to be highly unlikely because of the number and complexity of the steps that would need to take place consecutively. Also, the potential impact on human health, in the unlikely event of successful transfer of a functional antibiotic resistance *nptII* gene in Cowpea Event 245F to microorganisms in the human digestive tract, is considered negligible.

The risks posed by the possibility of horizontal gene transfer from genetically modified plants containing the *nptII* gene to bacteria in the human digestive tract are negligible. The probability

of such transfer is extremely remote; the relative impact if it were to occur is insignificant considering that the *nptII* gene is already widespread within the environment and microbial communities.

In the case of genetically modified plant events, such as 245F cowpea, where the *nptII* gene is interrupted with the CAT-1 intron, expression of this gene in bacteria is not possible thus removing any residual concerns related to the remote possibility of horizontal gene transfer.

Based on the findings above, the Board of the NBA concludes that there is a stable integration of a single copy of the inserted DNA in the plant genome for Cowpea Event 245F.

3.3.3 Stable Integration into the Plant Genome

Based on the results of Southern hybridization analysis provided by the applicant, Cowpea Event 245F contained a single copy of the pMB6 T-DNA integrated at a single site within the cowpea genome. The introduced DNA in 245F cowpea did not contain any sequences derived from the pMB6 plasmid backbone region.

Results of the nucleotide sequence analysis of the entire T-DNA insert within 245F cowpea confirmed the organization and overall integrity of the inserted DNA. With the exception of 41-bp and 26-bp truncations of the 5' and 3' termini, respectively, the nucleotide sequence of the inserted T-DNA within event 245F was identical to that in plasmid pMB6 used for transformation. The site of insertion of the T-DNA was mapped to an inter-genic region on chromosome 1, and there were no new novel open reading frames created as a consequence of the DNA insertion.

Also, based on the results of event-specific and *cry2Ab*-specific PCR analysis, the inserted DNA derived from plasmid pMB6 T-DNA was stably inherited within progeny plants spanning five generations 245F cowpea in direct line of descent from the original transformation event.

Additionally, analysis of a segregating population of BC1F1 that represented a backcross generation within the breeding program between event 245F (IT86D-1010) and genetically modified cowpea SAMPEA 20-T (Event 709A) confirmed that the introduced *cry2Ab* encoding gene segregated as a single genetic locus according to Mendelian rules of inheritance.

Based on the findings above, the Board of the NBA concludes that there is a stable integration of a single copy of the inserted DNA in the plant genome for Cowpea Event 245F.

4.0 Environmental Risk Assessment

4.1 Potential Weediness and/ or Invasiveness of Cowpea Event 245F

The applicant provided data on phenotypic and agronomic traits to establish that Cowpea Event 245F is not weedier or more invasive than conventional cowpea varieties.

Information provided by the applicant included studies that showed the establishment of feral populations of domesticated cowpea is theoretically possible. This has rarely been observed in Africa. There was also information that a few small transient feral populations had been reported in coastal Kenya, which were not seen in consecutive years. However, cowpea cultivars are generally not capable of creating long-lived seed banks in the soil because their seeds are permeable to water and lack dormancy. The domestication of cowpea has resulted in loss of many primitive traits typically associated with weediness, such as perenniality, fecundity, hairiness, small size of seeds and pods, hard seeds, pod shattering, and significant out-crossing.

The applicant conducted field studies on 245F cowpea and its near-isogenic non-genetically modified counterpart in multiple locations in Ghana and Nigeria. No significant differences in plant growth and morphology, reproductive biology characteristics, seed set or shattering, were

observed. This indicates no unintended change in the weediness or invasiveness of Cowpea Event 245F compared to the conventional cowpea cultivars.

Considering the above information, the Board of the NBA concludes that Cowpea Event 245F has no increased weediness or invasiveness potential compared to conventional cowpea varieties.

4.2 Potential of Pollen Mediated Gene Flow from Cowpea Event 245F to Wild Relatives

The applicant provided information on the reproductive characteristics of cowpea, being cleistogamous, producing viable pollens and receptive stigma before anthesis, meaning that cowpea reproduction is entirely via self-pollination. However, out-crossing mediated by insects can occur naturally in the field, and while different insect species visit cowpea flowers, not all are responsible for pollen movement associated with out-crossing. Only heavy insects such as honey bees and bumblebees could be responsible for insect vectored pollen movement because they can depress the wings of cowpea flowers and expose their stamens and stigmas for pollination. Out-crossing rates between cultivated cowpea varieties are low, ranging from 0.5-0.85 percent when cowpea was planted in alternate rows 1 meter apart, and between 0.01-0.13 percent when planted in concentric circles around a pollen source. There are no reports of natural hybridization between *V. unguiculata* and other *Vigna* species.

In addition, there have been no changes in the reproductive biology of cowpea as a consequence of the genetic modification resulting in Cowpea Event 245F. Parameters such as percent germination, days to 50 percent flowering, pods per plant, healthy seed per plant, and seed weight are not significantly different between Cowpea Event 245F and control (conventional cowpea).

Expression of the introduced *cry2Ab* and *nptII* genes in 245F cowpea will not alter the frequency or efficiency of horizontal gene transfer (HGT). The potential for HGT and any possible adverse outcomes has been reviewed in the literature and risk is negligible, due to the rarity of HGT events and because the gene sequences (i.e., *cry2Ab* and *nptII*) are already present in the environment and available for transfer via demonstrated natural mechanisms.

The Board of the NBA concludes that the potential risk of gene flow from Cowpea Event 245F to wild relatives is negligible.

4.3 Potential for Cowpea Event 245F to Become a Plant Pest

The intended effects of the introduced trait in Cowpea Event 245F are unrelated to plant pest potential, and cowpea is not considered to be a plant pest. Field observations of Cowpea Event 245F by the applicant did not reveal any modifications to disease and pest potential, other than to *Maruca* pod borer.

The Board of the NBA therefore concludes that Cowpea Event 245F does not display altered pest potential compared to conventional cowpea varieties, except for the intended resistance to Maruca pod borer.

4.4 Potential Impact of Cowpea Event 245F on Non-Target Species, Including Humans

The applicant provided information and findings from several studies on the environmental safety of Cry2Ab to non-target organisms, which were reviewed as appropriate. There are existing data on the environmental safety of Cry2Ab to non-target organisms, which are entirely sufficient to complete the environmental risk assessment of Cowpea Event 245F. The applicant's assessment on a range of non-target organisms revealed that environmental release is unlikely to result in altered impacts on interacting organisms, with the exception of target lepidopteran insect species (i.e., *Maruca vitrata*).

Furthermore, reviews of the environmental safety of Cry2Ab have concluded that, based on toxicity testing with a range of representative non-target organisms, cultivation of genetically modified plants expressing *cry2Ab* would not significantly affect the abundance of non-target arthropods in comparison with alternative insect management practices.

The Board of the NBA therefore concludes that, compared to conventional cowpea varieties, the environmental release of Cowpea Event 245F will not result in altered impacts on non-target organisms, including humans.

4.5 Potential Impact of Cowpea Event 245F on Biodiversity

Cowpea Event 245F has no new phenotypic characteristics that would extend its range beyond the current geographic range of cowpea cultivation in Ghana. The applicant has also provided information to demonstrate that the insect resistance trait in Cowpea Event 245F will not spread into or persist in unmanaged environments. Cowpea Event 245F does not pose an increased risk to interacting non-target organisms compared to its conventional counterparts.

The Board of the NBA concludes that the potential impact on biodiversity of Cowpea Event 245F is not different from that of conventional cowpea varieties.

4.6 Potential for Development of Resistant *Maruca* Population

The applicant provided information on the pod borer resistant Cowpea Event 245F which will not be placed on the market (commercialized) as a standalone product, hence there is no requirement for deployment of an insect resistance management (IRM) plan for this single event. Event 245F will only be used as a breeding parent in the development of new cowpea varieties containing the Cry2Ab and the Cry1Ab, derived from Event 709A, to provide the required modes of action against *M. vitrata.*

Considering the above information on development of resistant Maruca population, the Board concludes that Cowpea Event 245F does not pose a risk based on the intended use.

5.0 Food & Feed Safety Assessment

5.1 Potential Impact of Cowpea Event 245F on Food and Feed Nutrition

5.1.1 Nutritional Composition

Applicant's submission indicated that compositional analyses were performed to compare the concentrations of major nutrient components in samples of whole grain, leaves collected from Cowpea Event 245F, and control (conventional) cowpea grown at six different locations representing typical cowpea growing conditions in West Africa. Additionally, concentrations of key minerals were determined in samples of whole grain.

From sample analyses no consistent patterns emerged to suggest that biologically meaningful changes in composition or nutritive value of the leaves or grain had occurred as an unintended consequence of the genetic modification. The conclusion based on these data was that whole grain and leaves derived from 245F cowpea were compositionally equivalent to these same products from their conventional counterparts. All mean values were within the commercial ranges and literature values as well as within the normal variation for conventional cowpea.

5.1.2 Anti-Nutrients and Secondary Metabolites

Concentrations of phytic acid were determined in samples of whole grain collected from Cowpea Event 245F and conventional cowpea grown at six different locations representing typical cowpea growing conditions in Ghana and Nigeria.

The applicant provided evidence to show that from the combined-sites analyses across the six locations, there was a small but statistically significant decrease (ca. −5 percent) observed in mean phytic acid content of Event 245F grain samples compared to control samples (conventional cowpea), which is biologically insignificant. All mean values from the analyses were within the commercial ranges and literature values as well as within the normal variation for conventional cowpea.

The Board of the NBA concludes that, based on the evidence provided by the applicant, the nutritional composition of Cowpea Event 245F is substantially equivalent to that of conventional cowpea varieties.

5.1.3 Potential Impact of Cowpea Event 245F on Workers and Processors

The Cry2Ab protein is commonly found in bacteria and has been used in some previously authorized genetically modified crops like maize in some countries. The Cry2Ab binds to specific receptors on the brush border membrane of midgut epithelial cells found only in target lepidopteran insect species initiating subsequent reactions that result in cell lysis and septicemia causing insect death. This does not occur in mammals, other vertebrates, or other insects because they lack the specific receptors for Cry2Ab.

The Cry2Ab protein does not display significant amino acid sequence similarity to known or putative protein mammalian toxins or allergens and it is rapidly degraded in Simulated Gastric Fluid (SGF) containing pepsin, with more than 99 percent degradation within 30 seconds as assessed by SDS-PAGE and Western Immunoblot analysis. Furthermore, the Cry2Ab protein is not acutely toxic in mice at dosages up to 2,198 mg/kg body weight, the highest dosage tested. This information indicates that the Cry2Ab protein is unlikely to be a toxin or an allergen.

The NPTII protein has been used in some previously authorized genetically modified crops in some countries. Its known function in catalyzing the ATP-dependent phosphorylation of the 3'-hydroxyl group of the amino-hexose portion of certain aminoglycosides is not similar to the activities of known protein toxins. Bioinformatic analyses showed that the NPTII protein does not display significant amino acid sequence similarity to known or putative protein toxins or allergens and it is rapidly degraded in SGF containing pepsin and Simulated Intestinal Fluid (SIF) containing pancreatin. No intact NPTII or NPTII-derived fragments were detected after exposure to pepsin-containing SGF, or pancreatin-containing SIF as assessed by Western Immunoblot labeling following SDS-PAGE analysis. The NPTII protein is not acutely toxic in mice at dosages up to 5,000 mg/kg body weight, the highest dosage tested. These results indicate that the NPTII protein is unlikely to be a toxin or an allergen.

The Board of the NBA, based on the evidence provided, concludes that there is no potential impact of Cowpea Event 245F on workers and processors with respect to toxicity and allergenicity.

6.0 Socioeconomic Impact Assessment that could Inform Decision-making

6.1 Socioeconomic Costs, Benefits and Trade Implications

Ghana's annual estimated demand for cowpea is 169,000 tons. Meanwhile, the country produces only 57,000 tons each year. The deficit is met with imports from neighbouring West African countries, notably Burkina Faso, Nigeria, and Niger. Nigeria is the largest producer of cowpea

globally (i.e., 45% of world production) followed by Niger (15%) and Burkina Faso (6%). To further increase their market share in the subregion, Nigeria has approved the cultivation of GM cowpea with Burkina Faso expected to do the same on completion of regulatory studies as the market preference is for quality produce.

Ghana has a huge potential to become a major producer and exporter of cowpea. However, production of the crop is reduced with yield losses ranging from 20–80 percent as a result of insect pests. The primary pest is *Maruca* pod borer *(Maruca vitrata).* This pest is primarily controlled by the use of synthetic insecticides. However, the continued use and effectiveness of these insecticides have been constrained by factors including resistance build-up, cost of the insecticide and labour, lack of appropriate training, and availability of personal protective equipment. In addition, the use of these insecticides poses health risks to growers (producers), their families, communities, and the environment due to exposure to the insecticides and run-off into water bodies. Moreover, a low literacy rate affects the ability of many farmers to read and follow application instructions. Drudgery from the frequent use of knapsack for spraying and distances covered in doing so by an aging farming population are additional constraints.

Thus, adopting GM cowpea that is benign to the environment and gives higher yields than the current practice of insecticide control will have far reaching socioeconomic benefits in terms of food security, improved livelihoods, and trade. Ghana could produce more cowpea to feed its people and with a surplus for export to earn foreign exchange to develop the country.

6.2 Concerns on Monopoly of Seeds by Multinationals

The gene used to develop the GM cowpea was obtained royalty-free from Monsanto (now Bayer) through the African Agricultural Technology Foundation. This therefore means that, varieties developed from this technology will be a public good and will be owned by CSIR-SARI, Ghana.

6.3 Concerns on Reusability of Seeds

The Pod Borer Resistant cowpea under development are not hybrids. Thus, just like other open-pollinated varieties, farmers can save the GM cowpea seeds for planting the next season. It is recommended, however, as part of good agronomic practices for all crops (not just GMOs) that farmers buy certified seeds at least every 3 years to guarantee seed purity and maximize yields. CSIR-SARI has also been engaging in public education efforts with various stakeholders, including farmers and the media, to ensure informed decisions on technology adoption and husbandry practices.

7.0 Public Comment

On April 28, 2023, the NBA published a notice in the Government Gazette No. 75 of 2023 concerning an application for environmental release of Cowpea Event 245F in Ghana. Furthermore, the general public was informed about the same application through the newspapers (specifically the Daily Graphic and Ghanaian Times) on Thursday, June 22, 2023. A 60-day window was provided for the public to provide comments. However, no written comments were received by the NBA.

8.0 New Information Requirements

If at any time, the applicant becomes aware of any information regarding risk to health and the environment, which could result from release of Cowpea Event 245F in this country or elsewhere, the applicant shall immediately provide such information to the NBA. On the

basis of such new information, the NBA will re-evaluate the potential impact of Cowpea Event 245F and take appropriate measures.

9.0 Risk Management Requirements

9.1 Insect Resistance Management Plan

There is no requirement for the deployment of an insect resistance management (IRM) plan for this single event. Event 245F will only be used as a breeding parent in the development of new cowpea varieties containing the Cry2Ab (245F) and the Cry1Ab (709A), to provide the required modes of action against *M. vitrata.*

10.0 Conclusion

In reaching a final decision on this application, the Board took into account the following: information submitted by the applicant; the Technical Advisory Committee's (TAC) report on the review of the risk assessment; socioeconomic considerations that could arise from the introduction of the Cowpea Event 245F into the environment and found these to be adequate to make decision on the application for environmental release of Cowpea Event 245F.

11.0 Decision

The Board of the National Biosafety Authority (NBA), in light of the foregoing, approves the environmental release of Cowpea Event 245F to be used as a breeding parent together with previously authorized Event 709A (AAT-7O9AA-4) to create new combined-event or stack varieties expressing both the Cry1Ab and Cry2Ab insecticidal proteins.

This approval does not include the placement of Cowpea Event 245F and the resulting breeding stack on the market without prior approval from the NBA.

This approval is granted with effect from 31st October 2023 to 30th October 2026.

Signature and Date:

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Chief Executive Officer of the National Biosafety Authority

31st October 2023

Date

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Chairman, Board of the National Biosafety Authority

31st October 2023

Date