



DECISION DOCUMENT FOR CONFINED FIELD TRIAL OF GENETICALLY MODIFIED COWPEA

Tracking No: 2024-283- SARI-008-C

Date: 18th December 2024

Title: Decision of an Application from the Council for Scientific and Industrial Research, Savanna Agricultural Research institute (CSIR - SARI) for Confined Field Trial (CFT) of Genetically Modified Cowpea (*Vigna unguiculata L. Walp.*) Resistant to Cowpea field pest (*Maruca vitrata*) Event PP099-EV113 in Ghana.

Legislation

Pursuant to Sections 4, 11, 17, 19, 21 and 22 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 Maruca resistant Cowpea Event PP099-EV113. The Board of the NBA has determined that the genetically modified plant does not present an altered environmental risk concern in Ghana. The Board has therefore approved the confined field trial of Event PP099-EV113 - Cowpea (*Vigna unguiculata L. Walp.*) Genetically Modified for Resistance to Maruca Pod Borer (*Maruca vitrata*) for three (3) year period, renewable.

1.0 Application Summary

Cowpea (*Vigna unguiculata L. Walp.*) is one of the most important grain legumes in Sub-Saharan Africa, particularly in Ghana, where it serves as a critical source of protein for millions of people. Rich in protein, certain vitamins, and minerals, cowpea is vital for food security and nutrition, especially in regions where animal protein is scarce or too costly. In addition to its nutritional value, cowpea plays a crucial role in sustainable agriculture due to its ability to fix nitrogen in the soil, which improves soil fertility and reduces the need for synthetic fertilizers. However, cowpea production faces significant challenges, primarily due to its vulnerability to pests, particularly the *M. vitrata* (Maruca pod borer), a lepidopteran pest that can cause up to 80% yield loss in some regions.

To address this issue, cowpea has been genetically modified to develop resistance to *M. vitrata*, one of the most destructive pests affecting cowpea in West Africa. The Council for

Scientific and Industrial Research, Savanna Agricultural Research Institute (CSIR-SARI) has acquired a genetically modified cowpea event, PP099-EV113, which expresses the CryIIe1 protein, conferring resistance to the pod borer. The genetic modification that produced the cowpea event PP099-EV113 was carried out at the Wisconsin Crop Innovation Center (WCIC) in Madison, Wisconsin, USA. This modification enables the cowpea to produce a protein effective against lepidopteran insects, such as *M. vitrata*, thereby reducing their capacity to infest and damage cowpea plants.

The dual T-DNA binary transformation vector was constructed *de novo* using Golden Gate cloning, incorporating synthesised DNA fragments modified to eliminate restriction enzyme recognition sites (Engler *et al.*, 2008). It includes the *ColE1* origin of replication from pBR322 for growth in *E. coli* and the RK2 *traF* and *oriV* replication components for growth in *Agrobacterium* strains, as well as a bacterial *nptIII* selection cassette.

Two sets of T-DNA border repeats, one for each T-DNA, were included. Each T-DNA has a 204-base pair fragment from the Ri plasmid of *Agrobacterium rhizogenes* strain NCPPB2659 (pRi2659, GenBank accession EU186381) containing the right border repeat (Mankin *et al.*, 2007). The left border comprises a 500-base pair fragment from pRi2659 and a 456-base pair fragment from the Ri plasmid of strain 1724 (pRi1724, GenBank accession NC_002575). Tandem left border repeats reduce T-DNA read-through, streamlining the screening process for backbone-free events (Podervin *et al.*, 2006).

A bacterial expression cassette positioned between the T-DNAs contains the coral *AmiGFP* gene (Alieva *et al.*, 2008) under the control of the *E. coli lacZ* promoter, enabling visual selection in bacteria. These sequences are absent in plants.

T-DNA 1 includes a gene-of-interest expression cassette encoding a codon-optimised *Bacillus thuringiensis* CryIIe1 protein for soybean expression. This is driven by the *Arabidopsis thaliana* ubiquitin 10 (*UBQ10*) promoter and terminated by the RUBISCO SSU sequence from *Nicotiana tabacum* (Mazur and Chui, 1985). Synthesised DNA fragments were assembled into T-DNA 1 using a modified Golden Gate method (Weber *et al.*, 2011).

T-DNA 2 contains a plant-selectable marker expression cassette with the dual-targeted *spectinomycin adenyltransferase* gene (*aadA1*) (Scolfield *et al.*, 1994). This is driven by the soybean ubiquitin 3 (*UBQ3*) promoter (De La Torre and Finer, 2015) and terminated by the actin 7 (*ACT7*) 3' UTR. This cassette was assembled from *de novo* synthesised DNA fragments and inserted into T-DNA 2 using Golden Gate cloning

The proposed confined field trial (CFT) aims to assess the efficacy of this genetically modified cowpea in mitigating *M. vitrata* damage under field conditions in Ghana. In addition to pest resistance, the trial will evaluate other agronomic traits, including growth performance, yield, and any potential environmental interactions. The trial will also compare the performance of this genetically modified cowpea against traditional non-GM varieties. The goal is to ensure that this modified cowpea offers improved pest resistance without causing unintended harm to the environment, non-target species, or human health. Table 1 provides a summary of the genetically modified organism (GMO).

Table 1: Short Summary of the Genetically Modified Organism (GMO)

Identification of the Modified Plant	Cowpea Event PP099-EV113
Applicant	Council for Scientific and Industrial Research, Savanna Agricultural Research Institute (CSIR- SARI)
Plant Species	Cowpea. <i>Vigna unguiculata</i> L. Walp.
Modified Traits	Resistance to lepidopteran insect, <i>Maruca vitrata</i> .
Trait Introduction Method	<i>Agrobacterium tumefaciens</i> mediated transformation.
Purpose (s) of the Modified Plant	To reduce loss of plant yield resulting from the Maruca Pod Borer infestation. Additionally, the event will be used in stacked combinations to develop durable cowpea varieties resistant to both field and storage pests, enhancing agricultural productivity and food security.

2.0 Risk Assessment

2.1 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 evaluating plant growth and agronomic performance, including yield, to demonstrate that the genetic modification did not result in unexpected, unintended, negative effects relative to the unmodified parental control cowpea. The risk assessment review considered the potential of:

- Pollination leading to establishment of an unapproved gene in wild populations.
- Seed dispersal from the CFT site
- Seed dispersal during transport
- Pilfering of seed from CFT site

- Harvest of CFT materials for food and feed use
- Impact on non-target organisms

2.2 Potential of Pollination Leading to Establishment of an Unapproved Gene in Wild Populations

The applicant provided data on the biology and mechanism of pollen dispersal of the cowpea to establish that the cowpea event PP099-EV113 does not lead to unapproved gene in wild populations.

Cowpea is cleistogamous, producing viable pollens and receptive stigma before anthesis, meaning that cowpea reproduction is entirely via self-pollination. With the cultivated cowpea, there is no reported method of pollen dispersal for out-crossing because the anthers release pollen during the first half of the night when the flowers are still closed, and the pollen is sticky and heavy. Some out-crossing mediated by insects can occur naturally in the field, however, when different insect species visit cowpea flowers, not all are responsible for pollen movement associated with out-crossing. Only honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) are responsible for insect-vectored pollen movement because only such heavy insects could depress the wings of cowpea flowers and expose their stamens and stigmas for pollination. Out-crossing rates between cultivated cowpea varieties are low, ranging between 0.5–0.85 percent when cowpea was planted in alternate rows one meter apart, and between 0.01–0.13 percent when planted in concentric circles around a pollen source. There are no reports of hybridization between *V. unguiculata* and other *Vigna* species.

Considering the above information, the Board of the NBA concludes that the establishment of an unapproved gene in wild population is very low, less than 1% cross pollination. There are also no sexually compatible wild relatives.

2.3 Potential of Seed Dispersal from the CFT Site

The applicant provided information that domesticates usually have no effective seed dispersal mechanism, and thus differ from most wild plants. The modification of seed dispersal is one of the first steps in domestication, which is the case in cowpeas no less than in other crops. Certain changes have characterized the evolution of most seed crops, namely, loss of seed dispersal mechanisms, increase in seed and leaf sizes, development of determinate or compact growth habits, shifts in life cycle to annuality and shorter duration, changes from outbreeding to inbreeding and a general loss of sensitivity to the environmental signals that previously regulated development. Many of these changes have occurred in cowpeas. Cowpea seeds ingested by animals are easily digested because of their soft seed coats and therefore the dispersal of viable seeds via animal excrement is unlikely.

The Board of the NBA therefore concludes that, seed dispersal of the cowpea event PP099-EV113 from CFT site is low because pods have no significant shattering ability in cultivated cowpea.

2.4 Potential of Seed Dispersal During Transport

The applicant provided information that seeds of the regulated genetically modified cowpea event PP099-EV113 will be packaged in a sealed plastic bag at least 500- gauge (0.125 mm) thickness (primary container), inside a sealed metal or plastic secondary container. The metal or plastic container shall be capable of protecting the seeds and preventing spillage or escape. The metal or plastic container will then be placed in an enclosed sturdy outer shipping container made of corrugated fiberboard, corrugated cardboard, or other material of equivalent strength. The primary packaging materials (plastic bag) will be destroyed by incineration on the trial site. Secondary containers (metal or plastic containers) will be cleaned by hand on the trial site and verified to be free of any residual plant material prior to removal from the trial site and return to the CSIR-SARI Nyankpala and Manga research station.

The Board of the NBA considering the foregoing, concludes that seed dispersal of cowpea event PP099-EV113 during transport is unlikely.

2.5 Potential of Pilfering of Seed from CFT Site

The applicant provided information that the trial site will be fenced to prevent the removal of plant material from the trial site. The trial site will have a continuous twenty-four-hour (24/7) security guarding regime to prevent unauthorized entry and to monitor for any breaches in the integrity of the security fencing. Additionally, staff will be trained to understand the need to confine the seed.

Considering the information provided by the applicant above, the Board of the NBA concludes that the potential of pilfering of seeds from CFT site is low.

2.6 Potential of Harvest of CFT Materials for Food and Feed Use

The applicant stated that staff will be trained to understand the need to confine the seed. They also provided information that the trial site will be fenced and have a 24/7 security guarding regime to prevent the removal of plant material or unauthorized entry, and to monitor for any breaches from the trial site. In addition, the Trial-in-Charge will be responsible for ensuring any required monitoring and for the disposition and/or storage of harvested material. All activity will be supervised and recorded in the Compliance Document Binder by the Trial Manager.

The Board of the NBA concludes that the potential of harvest of CFT materials for food and feed use will be low.

2.7 Effects on Non-Target Organisms

The applicant indicated that the protein CryIIe1 is not toxic to humans or non-target organisms. The expression of the CryIIe1 protein is constitutive.

Based on the information provided by the applicant in the application and the report from the TAC, the Board of the NBA concludes that the potential of impact on non-target organisms is negligible.

3.0 Conclusion

In accordance with Section 21 of the Biosafety Act, 2011 (Act 831) the Board of the National Biosafety Authority has reviewed the application received from the Savannah Agricultural Research Institute (SARI) to conduct a confined field trial on Event PP099-EV113 - Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Maruca Pod Borer (*Maruca vitrata*) at Nyankpala and Manga, Tamale.

This review included a risk assessment that was carried out by the Technical Advisory Committee (TAC) in accordance with Section 28 sub-section (1) of the Act.

Based on the recommendations of Technical Advisory Committee (TAC), and the information provided by the applicant, the Board concludes that the Confined Field Trial with the Event PP099-EV113 (Tracking Number: 2024-283- SARI-008-C) can be carried out safely at the approved site. The Board requires that the applicant, in carrying out the confined field trial, adheres to the Terms and Conditions specified in the schedule attached to this approval. The applicant is required to keep on file all documentation and records related to the trial. These documents should be available for inspection for seven (7) years following trial completion.

4.0 Decision

The Board of the National Biosafety Authority (NBA) considering the foregoing, finds the proposed conduct of confined field trial of Event PP099-EV113 - Cowpea (*Vigna unguiculata* L. Walp.) genetically modified for resistance to Maruca Pod Borer (*Maruca vitrata*) at Nyankpala and Manga, a low-risk activity.

The Board therefore grants approval for the confined field trial of the genetically modified cowpea (*Vigna unguiculata* L. Walp. for a three (3) year validity period effective 18th December 2024 to 17th December 2027, with subsequent renewals being administrative-based. Further to that, and in line with paragraph 3.4 the Board grants clearance for the importation of the seeds for the purposes of the research activity during the permit duration,

subject to final approval from the Plant Protection and Regulatory Services Directorate of MOFA (PPRSD).

Kindly note that this permit is not transferable. Failure to adhere to the conditions specified in the attached schedule shall result in the withdrawal of the approval for the confined field trial.



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MR. KWAME DEI ASAMOAH - OKYERE
CHIEF EXECUTIVE OFFICER

18th December 2024

Date



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PROF. YAO TETTEY
BOARD CHAIRMAN

18th December 2024

Date



**THE SCHEDULE TO THE APPROVAL FOR CONFINED FIELD TRIAL OF
MARUCA RESISTANT COWPEA EVENT PP099-EV113 IN GHANA**

TRACKING NO: 2024-283- SARI-008-C

Terms and conditions for Confined Field Trial of Genetically Modified Cowpea in Ghana

Approval

Pursuant to Section 21 of the Biosafety Act, 2011 (Act 831), and on the basis of the information contained in the application 2024-283- SARI-008-C and the risk assessment report submitted by the TAC on the application, this approval is issued authorizing the Savannah Agricultural Research Institute to undertake Confined Field Trial of genetically modified cowpea resistant to Maruca pod borer (*Maruca vitrata*) at the approved site at Nyankpala and Manga, near Tamale subject to the following terms and conditions.

1.0 Authorisation and Compliance

No genetically modified cowpea plant material can be removed from the trial site, transported, planted, or distributed without explicit authorization by the National Biosafety Authority (NBA) unless it is authorised under this permit. Failure to comply with the conditions of an authorized release is a contravention of Section 41 of the Biosafety Act, 2011 (Act 831), which attracts punitive action.

2.0 Packaging and Storage

2.1 All genetically modified cowpea plant material including seeds intended for transportation must be packaged in at least two secure containers to prevent spillage, dissemination, or infestation.

2.2 These packages must be clearly labelled with the name of Event and marked "GM material", dated and the name and contact details of the person responsible for the confined field trial and the name and contact details of the person responsible for the confinement of the material.

2.3 GM cowpea plant material and seeds must be segregated and kept separately from non-GM cowpea to minimize mishandling or co-mingling.

3.0 Transportation



- 3.1 The transportation of genetically modified cowpea plant material must be done through the most direct and safe route including air transport if necessary.
- 3.2 Records of the dispatch and receipt of genetically modified cowpea plant material must be kept.
- 3.3 Records of seed quantities transported must be maintained and provided to NBA, when requested.
- 3.4 The CSIR-SARI should liaise with the Ghana Revenue Authority, Customs Division (GRA-Customs) for advice on the importation and clearance of the seeds on arrival at the port of entry.

4.0 Reproductive Isolation

- 4.1 Reproductive isolation should be provided by border rows and an isolation distance. One border row should be planted with a mixture of conventional cowpea varieties (to ensure flowers are present while the test plants flower) and should be planted at the same or higher density than the test plants and treated with the same agronomic inputs to ensure healthy growth.
- 4.2 The border rows should be monitored weekly until the end of flowering to ensure sufficient growth and flowering to act as a pollen trap for foraging insects.
- 4.3 An isolation distance of 10m outside the border rows should be cleared of cowpea plants prior to planting of the trial.
- 4.4 The isolation distance should be monitored at least once every four weeks to identify and remove any cowpea plant prior to flowering.
- 4.5 Should cowpea plants set seed in the isolation area before they are uprooted, the whole isolation area should be included in the post-harvest monitoring.

5.0 Preventing Dissemination of Plant Material

- 5.1 No plant material from these trials shall enter the human food or livestock feed chain. The trial site should be secured to discourage foraging and pilfering.
- 5.2 The site must be marked with signs indicating it as a CFT site.

- 5.3 Seeding, planting, site maintenance, and harvesting equipment must be cleaned of all residual plant material at the trial site prior to being moved to other locations to prevent dispersal of genetically modified plant material.
- 5.4 All staff members must be trained to comply with the terms and conditions of this approval and to understand why the materials cannot be used for food or feed at this stage in the research.
- 5.5 Personnel leaving the trial site must not carry any plant material with them unless it is authorized for transfer to a contained facility and is securely packaged, labelled, and recorded appropriately in the logbook.

6.0 Trial Boundary Marking

- 6.1 In addition to physical barriers, markers should be placed at all corners of the trial site to identify the confined field trial boundaries. The markers (*e.g.*, flags, corner posts.) should be conspicuous, identifiable, and in place for both the trial seasons and the post-harvest restriction period.

7.0 Fertilizer and Pesticide Application

- 7.1 If fertilizers and pesticides are used on the trial site that requires a temporary prohibition on entry into the site, a sign must be posted at the access to the trial indicating the date and time of spraying as well as the time until safe entry. This condition is intended to protect the health and safety of all the workers and inspectors. The use of fertilizers and pesticides must comply with existing regulations *i.e.*, Section 44, sub-section (1), (2), (3), (4), (5), and (6) of the Environmental Protection Agency Act, 1994 (Act 490) and the Plants and Fertilizer Act, 2010 (Act 803).

8.0 Harvesting and Seed Set

- 8.1 All seeds and propagation materials, including non-modified control materials, must be used for the intended purposes as stated in the application.

9.0 Disposal

- 9.1 All plant material harvested from the confined field trial that is not retained, must be destroyed by controlled burning to ashes or buried at a depth of at least one metre below the surface.
- 9.2 All non-modified seed and plant material harvested from trial sites must be treated the same way as the genetically modified material and destroyed on site.

10.0 Storage

- 10.1 All genetically modified cowpea seeds and planting materials retained from planting or harvesting must be stored in clearly labelled and secure containers (designed to minimize the potential for spillage, mixing, or dissemination) and kept separate from conventional seeds and plant materials. Appropriate records must be retained.
- 10.2 Each container of genetically modified cowpea planting material must be labelled with the inscription "Genetically modified- Do not use without authorization" and dated.
- 10.3 The storage area for genetically modified cowpea planting material must be labelled with the contact details of the Principal Investigator responsible for the research.
- 10.4 In the event of any accidental spillage, mixing or dissemination of material, corrective action must be initiated and documented immediately. This must be done in consultation with the biosafety officer / IBC while the National Biosafety Authority is notified by telephone and in writing within 24 hours.

11.0 Post-Harvest Period

- 11.1 The post-harvest period may be delayed if repeat cowpea trials with the same events are run on the same site for subsequent seasons.
- 11.2 Upon completion of the trial, the site must be put under irrigation to induce germination of all volunteer cowpeas.
- 11.3 Post-harvest monitoring must continue once a week for at least 5 weeks, or until no cowpea volunteers have been identified for two consecutive weeks. During this period all cowpea volunteers must be recorded, uprooted, and destroyed.
- 11.4 All volunteer cowpea plants should be treated as genetically modified and destroyed on site by desiccation and controlled burning to ashes. If volunteers set flowers or seed before being uprooted, the post-harvest monitoring period must be extended for another 5 weeks.

12.0 Monitoring

- 12.1 During the trial growing seasons, the isolation distance must be monitored weekly to

ensure that all cowpea plants are removed before the seed set. Records must be kept of this in-season monitoring of the isolation area.

- 12.2 During the post-trial growing season, the trial site and the additional 5-metre buffer zone must be monitored weekly. Records must be kept of the post-harvest monitoring of the trial site.

13.0 Accidental Release

- 13.1 In the case of accidental release of viable planting materials into the environment, recoverable seeds or seedlings must be collected, destroyed, and documented immediately, in consultation with the biosafety officer / IBC. The National Biosafety Authority must be notified by telephone and in writing within 24 hours.
- 13.2 All cowpea plants at the release site must be destroyed as described in section 9 (disposal) above. The release site must be marked and monitored weekly for five (5) weeks.

14.0 Records

- 14.1 A detailed trial logbook and a file must be kept at the trial site during the trial period. This book and file must contain the permit(s) and records of transport; storage; staff compliance training; planting; in-season crop treatments; border row and isolation area monitoring; early termination or harvest; equipment cleaning; retained materials; crop and seed destruction; post-harvest site management and monitoring; and accidental release management.
- 14.2 These records must be maintained by the applicant and be available for inspection for a minimum of seven years after the end of the post-harvest period.

15.0 Reporting

- 15.1 The National Biosafety Authority must be notified in writing or by e-mail at least 10 days before planting and at least 10 days before harvesting each trial on the site.
- 15.2 A short trial report summarizing the biosafety aspects of the completed trial(s), including any approved amendments to the original confinement conditions, must be sent to the NBA not more than three weeks after the end of the post-harvest period.

16.0 Transferability

This approval shall not be transferred to any other institute, event, or location. It covers only the Institutional Biosafety Committee of SARI and its operations at Nyankpala and Manga.

