



DECISION DOCUMENT FOR CONFINED FIELD TRIAL OF GENETICALLY MODIFIED PLANT

Tracking No: 2024-032-SARI-006-C

Date: 3rd June 2024

Decision on an Application from the Council for Scientific and Industrial Research, Savanna Agricultural Research Institute (CSIR-SARI) for Confined Field Trial of Cowpea (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Maruca Pod Borer (*Maruca vitrata*) and/ or Bruchid (*Callosobruchus maculatus*) in Ghana

Regulation

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 CSIR-SARI. This information addressed the safety of the Maruca and/ or Bruchid resistant cowpea. The Board of 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 cowpea (*Vigna unguiculata* L. Walp.) genetically modified for resistance to maruca pod borer (*Maruca vitrata*) and/ or bruchid (*Callosobruchus maculatus*) for three (3) year period, renewable.**

1.0 Table 1: Description of genetically modified events

	Event DPS-142	Event DPS-158	Event DPS-162	Event DPS-085
Category of Genetic Modifications	Insect Resistant	Insect Resistant	Insect Resistant	Insect Resistant
Introduced Gene(s)	<i>Cry1Ab, Cry2Ab and αAI-1</i>	<i>mCry1Ca, αAI-1 and Cry2Ab</i>	<i>mCry1Ca, αAI-1 and Cry1Ab</i>	<i>mCry1Ca</i>
Phenotype	Resistance to lepidopteran insect (<i>Maruca vitrata</i>), and Bruchid weevil (<i>Callosobruchus maculatus</i>).	Resistance to lepidopteran insect (<i>Maruca vitrata</i>), and Bruchid weevil (<i>Callosobruchus maculatus</i>).	Resistance to lepidopteran insect (<i>Maruca vitrata</i>), and Bruchid weevil (<i>Callosobruchus maculatus</i>).	Resistance to lepidopteran insect (<i>Maruca vitrata</i>)
Modification Method	<i>Agrobacterium</i> mediated transformation	<i>Agrobacterium</i> mediated transformation	<i>Agrobacterium</i> mediated transformation	<i>Agrobacterium</i> mediated transformation
Selection Method	Antibiotic resistance	Antibiotic resistance	Antibiotic resistance	Antibiotic resistance

2.0 Application Summary

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume widely consumed by about 120 million people worldwide. Cowpea seeds and fresh peas are rich sources of protein, certain minerals, and vitamins. Cowpea is cleistogamous, producing viable pollens and receptive stigma before anthesis, meaning that cowpea reproduction is entirely via self-pollination. There is no mechanical dispersion of pollen from the flowers of cultivated cowpeas because the anthers release pollen during the first half of the night when the flowers are still closed. Honey and bumble bees have been implicated in a very low degree of outcrossing, ranging between 0.5%–0.85% when cowpea was planted in alternate rows one (1) meter apart, and between 0.01%–0.13% when planted in concentric circles around a pollen source.

The transgenic cowpea was genetically modified to resist damage by *Maruca vitrata* and/or *Callosobruchus maculatus* (cowpea products that are resistant to both field and storage pest, respectively). The durability of *Maruca* resistance was improved by adding multiple modes of action and an added resistance to the cowpea weevil (*Callosobruchus maculatus*).

The applicant combines known genes for control of *Maruca* with the α AI-1 gene for bruchid control to enhance the durability of Pod Borer Resistant (PBR) cowpea, combined with protection against postharvest weevil losses. The genetically modified cowpea was produced using four (4) transformation events - **DPS-085, DPS-162, DPS-158, and DPS-142**. The applicant provided data on the identity of cowpea events, a detailed description of the transformation methods, data and information on the insertion sites, gene copy numbers, levels of gene expression in the plant, and the role of the inserted genes and regulatory sequences. The newly produced proteins were identified and characterized. The application contained a combination of *Cry* genes i.e., *Cry1Ab*, *Cry2Ab*, and *mCry1Ca*, all of which demonstrate control of *Maruca vitrata*; and alpha-amylase inhibitor (α AI-1) to control bruchids. The Authority had previously approved *Cry1Ab*, *Cry2Ab* and α AI-1 in cowpea events 709A, 245F, and CSI32, respectively.

This confined field trial (CFT) will be used to evaluate 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. There is only one CFT experimental design with two components: component 1: a confined field experiment to evaluate agronomic performance; and a component 2: a contained net house experiment to evaluate *Maruca vitrata* resistance under conditions of artificial infestation. Both trials will be conducted within the existing CFT site. Additionally, the trial will also be used to produce seed for postharvest storage assessment of resistance to bruchid damage and for compositional analysis of key nutrients.

2.1 Design of the Transforming Vector/Transforming DNA for each event.

2.1.1 EVENT DPS-142

The transforming vector, pDIICI-142, is a derivative of the pCAMBIA2300 binary vector, p5000 (Beyene *et al.*, 2017), which was modified by removing the *nptII* plant-selectable marker expression cassette and replacing it with the Cre recombinase gene driven by soybean heat-shock inducible promoter HSP17.3-B (Schoffl *et al.*, 1984), and the spectinomycin (SpcN) resistance selectable marker gene under the control of the soybean ubiquitin promoter (Che *et al.*, 2021). The green fluorescent protein (GFP) encoding sequence was also included for visual identification of

transformed plants (Haseloff *et al.*, 1997). In addition, the base vector was designed to include restriction endonuclease sites and sequences to facilitate Golden Gate cloning (Engler *et al.*, 2008) of the three gene-of-interest expression cassettes for production of Cry1Ab, α AI-1, and Cry2Ab. The first expression cassette contained the *cry1Ab* gene under the control of the *Arabidopsis thaliana* ubiquitin 10 (UBQ10) promoter and actin 2 3' untranslated region (UTR). The second cassette contained the α AI-1 gene driven by the seed-specific PHA-L promoter, and the PHA-L 3' UTR, all derived from *Phaseolus vulgaris*. Finally, the third cassette contained the *cry2Ab* gene under the control of the *A. thaliana* ribulose-1,5-bisphosphate carboxylase (RUBISCO) small subunit (SSU) promoter (Krebbers *et al.*, 1988) and the termination sequence of the RUBISCO SSU from *Nicotiana tabacum* (Mazur and Chui 1985). Plasmid pDIICI-142 was introduced into *Agrobacterium tumefaciens* strain LBA4404 and used to transform cowpea embryonic axis explants using the method of Che *et al.*, (2021).

2.1.2 EVENT DPS-158

A derivative of the pCAMBIA2300 binary vector, p5000 (Beyene *et al.*, 2017), was modified by removing the *nptII* plant-selectable marker expression cassette and replacing it with the Cre recombinase gene driven by soybean heat-shock inducible promoter HSP17.3-B (Schoffl *et al.*, 1984), and the spectinomycin (SpcN) resistance selectable marker gene under the control of the soybean ubiquitin promoter (Che *et al.*, 2021). The gene encoding the red fluorescent protein (DsRed2) from *Discosoma sp.* under control of the soybean elongation factor 1A (EF1A) promoter was also included for visual identification of transformed plants (Wasson-Blader 2001). In addition, the base vector was designed to include restriction endonuclease sites and sequences to facilitate Golden Gate cloning (Engler *et al.*, 2008) of the three gene-of-interest expression cassettes for production of mCry1Ca, α AI-1, and Cry2Ab. The first expression cassette contained the *mcry1Ca* gene under the control of the *Arabidopsis thaliana* ubiquitin 10 (UBQ10) promoter and actin 2 3' untranslated region (UTR). The second cassette contained the α AI-1 gene driven by the seed-specific PHA-L promoter, and the PHA-L 3' UTR, all derived from *Phaseolus vulgaris*. Finally, the third cassette contained the *cry2Ab* gene under the control of the *A. thaliana* ribulose-1,5-bisphosphate carboxylase (RUBISCO) small subunit (SSU) promoter (Krebbers *et al.*, 1988) and the termination sequence of the RUBISCO SSU from *Nicotiana tabacum* (Mazur and Chui 1985). Plasmid pDIICI-158 was introduced into *Agrobacterium tumefaciens* strain LBA4404 and used to transform cowpea embryonic axis explants using the method of Che *et al.*, (2021). Expression of the α AI-1 protein is controlled by the seed-specific *leucoagglutinating phytohemagglutinin* (PHA-L; *dlec2*) promoter from *Phaseolus vulgaris*.

2.1.3 EVENT DPS 162

A derivative of the pCAMBIA2300 binary vector, p5000 (Beyene *et al.*, 2017), was modified by removing the *nptII* plant-selectable marker expression cassette and replacing it with the Cre recombinase gene driven by soybean heat-shock inducible promoter HSP17.3-B (Schoffl *et al.*, 1984), and the spectinomycin (SpcN) resistance selectable marker gene under the control of the soybean ubiquitin promoter (Che *et al.*, 2021). The gene encoding the red fluorescent protein (DsRed2) from *Discosoma sp.* under control of the soybean elongation factor 1A (EF1A) promoter was also included for visual identification of transformed plants (Wasson-Blader 2001). In addition, the base vector was designed to include restriction endonuclease sites and sequences to

facilitate Golden Gate cloning (Engler *et al.*, 2008) of the three gene-of-interest expression cassettes for production of mCry1Ca, α AI-1, and Cry1Ab. The first expression cassette contained the *mcry1Ca* gene under the control of the *Arabidopsis thaliana* ubiquitin 10 (UBQ10) promoter and actin 2 3' untranslated region (UTR). The second cassette contained the α AI-1 gene driven by the seed-specific PHA-L promoter, and the PHA-L 3' UTR, all derived from *Phaseolus vulgaris*. Finally, the third cassette contained the *cry1Ab* gene under the control of the *A. thaliana* ribulose-1,5-bisphosphate carboxylase (RUBISCO) small subunit (SSU) promoter (Krebbers *et al.*, 1988) and the termination sequence of the RUBISCO SSU from *Nicotiana tabacum* (Mazur and Chui, 1985). Plasmid pDIICI-162 was introduced into *Agrobacterium tumefaciens* strain LBA4404 and used to transform cowpea embryonic axis explants using the method of Che *et al.* (2021).

2.1.4 EVENT DPS 085

A derivative of the pCAMBIA2300 binary vector, p5000 (Beyene *et al.*, 2017), was modified by removing the *nptII* plant-selectable marker expression cassette and replacing it with the Cre recombinase gene driven by soybean heat-shock inducible promoter HSP17.3-B (Schoffl *et al.*, 1984), and the spectinomycin (SpcN) resistance selectable marker gene under the control of the soybean ubiquitin promoter (Che *et al.*, 2021). The gene encoding the red fluorescent protein (DsRed2) from *Discosoma sp.* under control of the soybean elongation factor 1A (EF1A) promoter was also included for visual identification of transformed plants (Wasson-Blader 2001). In addition, the base vector was designed to include restriction endonuclease sites and sequences to facilitate Golden Gate cloning (Engler *et al.*, 2008) of the gene-of-interest expression cassette for production of mCry1Ca. The expression cassette contained the *mcry1Ca* gene under the control of the *Arabidopsis thaliana* actin 2 (ACT2) promoter and ACT2 3' untranslated region (UTR). Plasmid pDIICI-085 was introduced into *Agrobacterium tumefaciens* strain LBA4404 and used to transform cowpea embryonic axis explants using the method of Che *et al.* (2021).

The various Cry proteins measured in leaf tissue samples from different generations of transgenic events is presented in Table 2. Concentrations were determined by specific quantitative enzyme linked immunosorbent assay (ELISA) for mCry1Ca, Cry1Ab, and Cry2Ab, as appropriate for each event. DPS-0851 produces only mCry1Ca, DPS-142 series events produce only Cry1Ab and Cry2Ab in the leaf tissue, DPS- 158 series events produce only mCry1Ca and Cry2Ab in leaf tissues, and DPS-162 series events produce only mCry1Ca and Cry1Ab in leaf tissues. ND= Not done.

2.2 Table 2: Concentrations of various Cry proteins measured in leaf tissue samples from different generations of transgenic events

Event	Generation	Zygosity	Reps	Concentration (ng/mg FWT)		
				mCry1Ca	Cry1Ab	Cry2Ab
DPS-08501	T1	Hemizygous	5	8.49 $\pm$ 3.2		
	T2	Homozygous	41	18.8 $\pm$ 9.3		
	T3	Homozygous	10	17.4 $\pm$ 0.9		

DPS-14208	T1	Hemizygous	1		1.48	870
	T2	Homozygous	30		2.92 ± 1.97	1622 ± 291
	T3	Homozygous	100		5.25 ± 1.68	1572 ± 219
DPS-14217	T0	Hemizygous	1		ND	860
	T1	Homozygous	22		4.00 ± 2.42	1079 ± 353
	T2	Homozygous	8		ND	981 ± 324
DPS-15807	T0	Hemizygous	1	27.2		590
	T1	Hemizygous	9	20.0 ± 4.6		568 ± 319
	T2	Homozygous	44	32.6 ± 7.3		1867 ± 217
DPS-15811	T0	Hemizygous	1	17.3		850
	T1	Hemizygous	11	14.9 ± 7.1		603 ± 305
	T2	Homozygous	55	23.3 ± 9.8		1685 ± 416
DPS-16214	T0	Hemizygous	1	32.4	4.37	
	T1	Homozygous	3	22.7 ± 1.7	7.77 ± 3.8	
	T2	Homozygous	29	24.0 ± 6.4	8.47 ± 5.3	
DPS-16217	T0	Hemizygous	1	28.7	5.92	
	T1	Homozygous	11	18.4 ± 1.7	11.0 ± 4.1	
	T2	Homozygous	36	25.7 ± 5.5	8.38 ± 3.8	
DPS-16235	T0	Hemizygous	1	25.2	5.72	
	T1	Homozygous	4	35.6 ± 13.6	9.13 ± 6.3	
	T2	Homozygous	24	25.5 ± 4.8	6.04 ± 1.1	

3.0 Risk Assessment

3.1 Criteria

The Board of the NBA reviewed the risk assessment report from the 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

3.2 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 events will 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.

3.3 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 events from CFT site is negligible.

3.4 Potential of Seed Dispersal During Transport

The applicant provided information that seeds of the regulated genetically modified cowpea event DPS 08501 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 research station.

The Board of the NBA considering the foregoing, concludes that seed dispersal of cowpea events during transport is unlikely.

3.5 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.

3.6 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 is unlikely.

3.7 Potential of Impact on Non-Target Organisms

Cowpea is attacked by numerous insect pests during all stages of its growth and development. Insect pests include species from the following orders: Lepidoptera, Hemiptera, Coleoptera and Thysanoptera (Singh *et al.* 1990). The Cry1Ab, Cry2Ab, and mCry1Ca proteins expressed in Bt-cowpea specifically target lepidopteran pests (CERA 2011, 2013; OECD 2007). Accordingly, they are not expected to affect other non-lepidopteran pests such as thrips, pod-sucking bugs, and aphids. The applicant indicated that the proteins α AI-1, Cry1Ab, Cry2Ab and mCry1Ca are not toxic to humans or non-target organisms (NTOs). The α AI-1 protein is expressed only in the seeds of cowpea events. It is identical to the α AI-1 protein produced in the common bean, *Phaseolus vulgaris*, which is grown on more than four million hectares annually in Africa and has not been associated with adverse impacts on NTOs. The weight of evidence shows that Cry1A and Cry2A toxins have a very narrow activity spectrum targeting insects in the order Lepidoptera (OECD 2007; CERA 2010, 2011, 2013). Safety assessments of the Cry1A (both Cry1Ab and Cry1Ac) and Cry2A toxins have shown that their insecticidal activity is highly specific for lepidopterans (Huesing *et al.* 2011; CERA 2010, 2011, 2013). The toxicity and specificity of the Cry1A and Cry2A proteins are associated with their solubilization and proteolytic activation in the digestive tracts of susceptible lepidopteran insects.

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

4.0 Conclusion

In accordance with Section 21 of the Biosafety Act, 2011 (Act 831) the Board of the NBA has reviewed the application received from CSIR-SARI to conduct a confined field trial of cowpea (*Vigna unguiculata* L. Walp.) genetically modified for resistance to maruca pod borer (*Maruca vitrata*) and/ or bruchid (*Callosobruchus maculatus*) in Ghana.

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

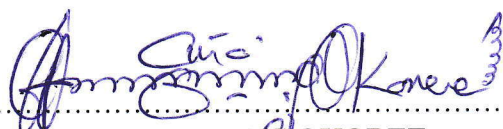
Based on the recommendations from TAC, and the information provided by the applicant, the Board concludes that the confined field trial with the tracking Number: 2024-032-SARI-006-C can be carried out safely at the approved site. The Board requires that the applicant, in carrying out the confined field 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 at least seven (7) years following trial completion.

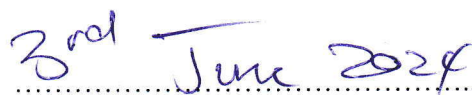
5.0 Decision

The Board of the National Biosafety Authority (NBA) in light of the foregoing, finds the proposed conduct of confined field trial of cowpea (*Vigna unguiculata* L. Walp.) genetically modified for resistance to maruca pod borer (*Maruca vitrata*) and/ or bruchid (*Callosobruchus maculatus*) could be carried out at the research station at Nyankpala

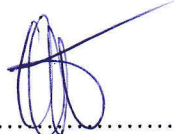
The Board therefore grants approval for the confined field trial of the cowpea (*Vigna unguiculata* L. Walp. for a three (3) year validity period effective 3rd June 2024 with subsequent renewals being administrative-based. Further to that, and in line with paragraph 3.4 the Board grants clearance for 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) based on the Plant and Fertilizer Act 2010, (Act 803).

Kindly note that this permission 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.


.....
MR. ERIC AMANING OKOREE
CHIEF EXECUTIVE OFFICER


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Date


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PROF. YAO TETTEY
BOARD CHAIRMAN

Date



**THE SCHEDULE TO THE APPROVAL FOR CONFINED FIELD TRIAL OF MARUCA
AND/ OR BRUCHID RESISTANT COWPEA EVENTS IN GHANA:**

TRACKING NO: 2024-032-SARI-006-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 2024-032-SARI-006-C and the risk assessment report submitted by the TAC on the application, this approval is issued authorizing the CSIR-SARI to undertake confined field trial of genetically modified cowpea resistant to Maruca pod borer (*maruca vitrata*) and/ or Bruchid (*Callosobruchus maculatus*) at the approved site at Nyankpala, 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 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 inscription “GM material”, dated and the name and contact details of the person responsible for the confined field trial and the confinement 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 is to liaise with the Ghana Revenue Authority, Customs Division (GRA-Customs) and Plant Protection and Regulatory Services Directorate (PPRSD) 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.
- 4.4 The isolation distance should be monitored at least once every four weeks to identify and remove cowpea plants 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 that the cowpea plants shall not be used for animal feed or food.
- 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 and labelled and recorded appropriately in the log-book.

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 fertilizer and pesticide 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 re-entry period. This condition is intended to protect the health and safety of the inspection staff. The use of fertilizer and pesticide 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 The trial may be terminated before the onset of flower development. If not, all seed and propagation materials, including non-modified control materials, must be destroyed on the site, unless authorized and NBA permission has been obtained to keep plant materials for further testing or seed for subsequent study or use in subsequent authorized planting(s) under CFT guidelines.

9.0 Disposal

- 9.1 All primary packaging materials for imported seeds must be destroyed by controlled burning to ashes.
- 9.2 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.
- 9.3 All non-modified seed and plant material harvested from trial sites must be considered genetically modified 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, including the contact details of the Principal Investigator responsible for the research.
- 10.4 In the event of any accidental spill, 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

immediately 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 zone must be monitored weekly (refer to section 11 on "Post-harvest period"). Records must be kept of the post-harvest monitoring of the trial site.

13.0 Unintended Release

- 13.1 In the case of unintended 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 and the Environmental Protection Authority (EPA) must be notified by telephone immediately and in writing within 24 hours.
- 13.2 All cowpea plants at the release site must be destroyed as described in section 9 above. The release site must be marked and monitored weekly.

14.0 Records

- 14.1 A detailed trial logbook and a file must be kept. 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 unintended release management.
- 14.2 These records must be maintained by the applicant and be available for inspection for at least 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 CSIR-SARI and its operations at Nyankpala.