

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

Date: 29TH MAY 2024

Title: Review of 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.

Introduction

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.

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

Derivation of the Transforming Vector/Transforming DNA for each event.**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).

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*.

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).

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.

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 ± 3.2		
	T2	Homozygous	41	18.8 ± 9.3		
	T3	Homozygous	10	17.4 ± 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	

The risk matrix for the assessment is presented in Table 3.

Table 3: Risk matrix for the assessment

Concern	Hazard	Likelihood	Consequence	Risk Management	Acceptable risk?
Pollination leading to establishment of an unapproved gene in wild populations	Environmental hazard not yet confirmed but believed to be unlikely. Human health hazard very low.	Very low (less than 1% cross pollination; no sexually compatible wild relatives)	Negligible – genes will dilute out of wild populations in the absence of cultivation	Keep other cowpea trials out of the isolation zone around the CFT. Enforce 10m isolation distance and in addition, use border rows to catch pollen from pollinators. Report any incidents.	Yes, with isolation zone and border rows.
Seed dispersal from the CFT site	Release of an unapproved GM material through natural seed	Low (pods have no significant shattering ability in	Negligible – small numbers of GM cowpea plants will dilute out of wild	Good stewardship practice to prevent pilferage of	Yes, with good stewardship along the production

	dispersal agents.	cultivated cowpea)	populations in the absence of cultivation	seeds from CFT site. Manage harvest to minimise seed dispersal. Monitor and devitalize volunteers for adequate period after last planting. Report any incidents.	chain and post-harvest monitoring.
Seed dispersal during transport	Release of an unapproved GM material through accident.	Low (Hardly any reported spillage of seed during transport by air or road)	Negligible – small numbers of GM cowpea plants will dilute out of wild populations in the absence of cultivation	Record movement of seed. Transport in three layers of packaging. Have a contingency plan for unintended release.	Yes, with recording, transport requirements, and contingency plan.
Pilfering of seed from CFT site	Exposure of an unapproved GM material to the public.	Low	Low- cost of removing unapproved seed from local use and in unapproved sites.	Control access to the site by keeping the site away from unauthorized persons. Staff training to explain the need to confine the seed.	Yes, with workers educated on good stewardship practices.

Harvest of CFT materials for food and feed use	Consumption of unapproved GM material.	Low	Negligible – proteins are known to be safe to consume.	Control access to the site by keeping the site away from unauthorized persons. Staff training to explain the need to confine the seed.	Yes, with workers educated on good stewardship practices.
Impact on non-target organisms	Possible decrease of other insect populations that feed on cowpea.	Low (gene expression in this experiment are seed specific.	Negligible for a CFT where size is too small for permanent impact	None needed	Yes (expected outcome would be desirable)

Recommendation

The Technical Advisory Committee (TAC) concludes that this is a low-risk activity, having an acceptable level of risk for Ghana, if the recommended risk management conditions are implemented.

**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.

