National Biosafety Authority

DECISION DOCUMENT FOR CONFINED FIELD TRIAL OF GENETICALLY MODIFIED PLANT

Tracking No: 2023-030-SARI-005-C **Date: June 21, 2023**

Decision on an Application from the Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (CSIR-SARI) for the Confined Field Trial of Cowpea Event CSI32 (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Bruchid (*Callosobruchus maculatus*) at Nyankpala, Tamale.

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 i.e. the Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (CSIR-SARI). This information addressed the safety of the Bruchid resistant Cowpea Event CSI32. 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 Cowpea Event CSI32 (*Vigna unguiculata* L. Walp.) Genetically Modified for Resistance to Bruchid (*Callosobruchus maculatus*) for a three (3) year period, renewable.**

1.0 Short Summary of the Genetically Modified Organism (GMO)

Identification of the Modified Plant	Cowpea Event CSI32
Applicant	Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (SARI)
Plant Species	Cowpea. *Vigna unguiculata* L. Walp.
Modified Traits	Bruchid resistance
Trait Introduction Method	*Agrobacterium tumefacians* mediated transformation.
Purpose (s) of the Modified Plant	To reduce post-harvest losses resulting from the Bruchid pest.

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 a rich source of protein, certain minerals, and vitamins. Cowpea seeds are highly susceptible to the storage pest, Bruchid (Cowpea weevil) which causes massive damage to the stored seeds and seriously limits its potential. Success through conventional breeding methods for developing resistant varieties is limited due to the natural genetic barriers in cowpea germplasm. Consequently, the transfer of insect pest resistance genes by genetic engineering could potentially aid plant breeders in overcoming these constraints.

In line with this, the CSIR-SARI has acquired the Bruchid resistant Cowpea Event CSI32 sourced from the Commonwealth Scientific and Industrial Research (CSIRO), Canberra, Australia. This is a transgenic line resistant to *Callosobruchus maculatus* (Cowpea weevil), intended to reduce post-harvest losses resulting from this devastating storage pest.

Transgenic Cowpea Event CSI32 was produced by *Agrobacterium* mediated transformation and the selection method used in plant regeneration was herbicide tolerant. A bar gene expression cassette consisting of the 35S promoter from Cauliflower Mosaic Virus (CaMV), the bar gene from *Streptomyces hygroscopicus*, and the 3' untranslated region of the *A. tumefaciens* Ti plasmid octopine synthase (OCS) derived from plasmid pSLJ2011 (Jones *et al.*, 1992) was inserted into the binary vector pPZP200 (Hajdukiewicz *et al.*, 1994) to yield the binary vector pPOPBar. At the time of insertion, the CaMV 35S promoter was truncated at the ScaI site to reduce the size from 1395 bp to 698 bp. An *Arabidopsis thaliana* putative matrix attachment region (MAR; 2.3kb) from plasmid pJW32 (Liu and Tabe, 1998) was inserted at 3' to the bar gene cassette. A second pair of border sequences, amplified from plasmid pPZP200 by PCR, was inserted at the 3' end of the MAR sequence, to give a twin binary construct pPOPbarRBLB. Finally, the α-amylase inhibitor (αAI-l) gene cassette, consisting of the PHA-L (*dlec2*) promoter from *Phaseolus vulgaris*, the bean αAI-l gene, and the PHA-L 3' untranslated region, was isolated from plasmid pTA3 (Altabella and Chrispeels, 1990) and inserted downstream of the MAR sequence at the *Hind*lll site between the extra right and left borders to yield the binary plasmid pPOP3.3 used to transform cowpea.

The bar gene expression cassette, which was contained in an independent T-DNA segment, was removed by genetic segregation during the breeding of Cowpea Event CSI32. Thus, the only newly expressed protein in Cowpea Event CSI32 is the alpha-amylase inhibitor derived from the common bean, *Phaseolus vulgaris.*

The CSIR-SARI provided data on the identity of Cowpea Event CSI32, 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 were identified and characterized. There were also no indications that the Cowpea Event CSI32 would be more invasive or persistent in the environment or have altered susceptibility to pests and diseases.

The purpose of the Confined Field Trial (CFT) is to evaluate the efficacy of the Cowpea Event CSI32, assess plant growth and agronomic performance including yield, and demonstrate that the genetic modification did not result in any unexpected, unintended, negative effects relative to the unmodified parental control cowpea. The trial will also be used to produce seed (grain) for postharvest storage and for compositional analysis of key nutrients.

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

- Pollination leading to the 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 the 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 CSI32 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 from 0.5--0.85 percent when cowpea was planted in alternate rows one meter apart, and from 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 establishment of an unapproved gene in the wild population in Ghana is very low, with less than 1% cross-pollination. There are also no sexually compatible wild relatives in Ghana. Therefore, the Board concludes that there is no potential for pollination.

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, and 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 the 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.

Seed dispersal of the Cowpea Event CSI32 from the CFT site is low because pods have no significant shattering ability in cultivated cowpea. Therefore, the Board concludes that there is no potential for seed dispersal.

3.4 Potential of Seed Dispersal During Transport

The applicant provided information that seeds of the regulated Genetically Modified Cowpea Event CSI32 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 will 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 bags) will be destroyed by incineration on the trial site. Secondary containers (metal or plastic containers) will be cleaned 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.

Seed dispersal of Cowpea Event CSI32 during transport is unlikely. Considering the foregoing, the Board concludes that there is no potential for seed dispersal during transport.

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 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 concludes that the potential of pilfering seeds from the 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 harvest of CFT materials for food and feed use will be low or unlikely Therefore the Board concludes that there is no potential for the harvest of CFT materials.

3.7 Potential of Impact on Non-Target Organisms

The applicant indicated that the protein α-amylase inhibitor-l (αAI-l) is not toxic to humans or non-target organisms. The αAI-l protein is expressed only in the seeds of Cowpea Event CSI32 and is identical to the αAI-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 non-target organisms (NTOs). α-amylase inhibitors occur in many plants as part of the natural defense mechanisms. They are particularly abundant in cereals and legumes. The activity of the bean AaI-1 protein is specific in inhibiting the growth and development of certain granivorous insect species, such as *Callosobruchus chinensis* (adzuki bean weevil) and *C. maculatus*, but not *Zabrotes subfaciatus* (Mexican bean weevil) or the coleopteran cotton boll weevil (*Anthonomus grandis*). Although αAI-1 can inhibit the α-amylases of some members of Hymenoptera and Diptera orders, the seed-specific expression of αAI-1 in Cowpea Event CSI32 means there is no route of potential exposure to beneficial insect species like honeybees. The production of transgenic plants expressing α-amylase inhibitors is an attractive and alternative eco-friendly approach in comparison to the use of chemical pesticides and insecticides that could contribute to developing resistant crop varieties to their major target insect pests.

Impact on non-target organisms is negligible for such a CFT where the size is too small for permanent impact. The Board concludes that there is no route of potential exposure to beneficial insect species.

4.0 Conclusion

In accordance with Section 21 of the Biosafety Act, 2011 (Act 831), the Board of the National Biosafety Authority (NBA) has reviewed the application received from the Savannah Agricultural Research Institute (SARI) to conduct a Confined Field Trial on Cowpea Event CSI32 (*Vigna unguiculata* L. Walp.) Genetically Modified for resistance against Bruchid (*Callosobruchus maculatus*) at Nyankpala, Tamale.

Based on the review of the above criteria in section 3.1, the potential of cross-pollination, seed dispersal (from the site or during transport), and the use of the seeds for food and feed are low. Equally, the impact of Cowpea Event CSI32 on non-target organisms is negligible. The Board, therefore, concludes that the Confined Field Trial of the Cowpea Event CSI32 (Tracking Number: 2023-030-SARI-005-C) can be carried out safely at the approved site.

5.0 Decision

The Board of the National Biosafety Authority (NBA) in light of the foregoing, finds the proposed conduct of the Confined Field trial of the Cowpea Event CSI32 (*Vigna unguiculata* L. Walp.) Genetically Modified for resistance to Bruchid (*Callosobruchus maculatus*) at Nyankpala, a low-risk activity.

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 June 21 2023 with subsequent renewals being administrative.

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). The CSIR-SARI is to liaise with the Ghana Revenue Authority, Customs Division (GRA-Customs) for advice on the importation and clearance of the seeds at the port of entry.

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 the trial completion.

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.

.....

MR. ERIC AMANING OKOREE
CHIEF EXECUTIVE OFFICER

21 June, 2023
.....
Date

.....

PROF. CHARLES ANTWI-BOASIAKO
BOARD CHAIRMAN

21 JUNE 2023
.....
Date

NATIONAL BIOSAFETY AUTHORITY NBA GHANA