REPORT OF THE NATIONAL BIOSAFETY COMMITTEE (NBC) ON ASSESSMENT OF THE APPLICATION BY THE INSTITUTE FOR AGRICUTURAL RESEARCH (IAR), ZARIA, FOR A BIOSAFETY PERMIT FOR CONFINED FIELD TRIAL OF *TRANSGENIC COWPEA*, HELD AT SOUNDER'S SUITES, PLOT 1269 BAMANGA TUKUR STREET, APO LEGISLATIVE QUARTERS, ZONE E, 900104, ABUJA ON THE 26-27TH JANUARY, 2023.

INTRODUCTION

In line with the National Biosafety Management Agency (NBMA) regulations, an ad-hoc National Biosafety Committee (NBC) was constituted by the DG/CEO, NBMA under the Chairmanship of Professor Celestine Aguoru, with the list of NBC members attached.

The Committee in expressing her opinion relied on the dossier submitted by the applicants, NBTS document, expertise of the members and other relevant documents with the aim of advising the Agency on the merits and demerits of the application.

Mode of Assessment

The application was assessed through an in-depth review of the submitted dossier

S/ No		OBSERVATIONS	REMARKS/ RECOMMENDATION
1.	**Administrative Information**		
	Purpose of Application	The purpose of the CFT is to collect agronomic and phenotypic data during the growing season and yield at harvest, and to evaluate postharvest resistance to bruchid damage at the Institute for Agricultural Research (IAR) of Ahmadu Bello University (ABU) Zaria.	
	Previous applications or approvals	This is a new application. No applications for CFTs of the cowpea event described herein have previously been submitted in Nigeria. The cowpea event described herein has not been authorized for commercial release in any jurisdiction, nor have any applications for CFTs been denied in any jurisdiction.	
	Applicant	Institute of Agricultural Research (IAR), Zaria	
	Contact Details of Principal Investigator	Contact Details of Principal Investigator: Name of Lead Scientist: Prof. Mohammad F. Ishiyaku Institute for Agricultural Research, Ahmadu Bello University PMB 1044 Zaria, Nigeria Tel: +234 8069299881 E-mail: mfishiyaku@abu.edu.ng ; mffaguji@hotmail.com **Trial manager:** Dr. Muhammad Lawan Umar **Address:** Dept. of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, PMB 1044 Zaria, Nigeria **E-mail:** mlumar@abu.edu.ng ; mahammadlawan@yahoo.com **Telephone:** +234 806 919 8100	The Curriculum Vitae of the Principal Investigator and the Trial Manager were not attached
	Proposed Location and Size of Trial	**Trial Site Location:** Institute for Agricultural Research (IAR) Research Farm Km 17 along Zaria-Sokoto Road, Zaria, Kaduna State. **Agroecological Zone:** Northern Guinea Savanna.	Be consistent with your units of measurements.

		GPS Coordinates: 11º 10.860[1] N, 7º 36.910[1] E. **Size**: The trial dimensions will be 15.25m x 37.5m for a total area of 571.9 m^2, excluding the spatial isolation distance. The CFT facility is secured with a 6 ft high chain-link wire fence to keep out unauthorized personnel and/or livestock. A map of the trial site is provided in Annex 1 (page **Error! Bookmark not defined.**).	
	Proposed duration of Trial	**Proposed Duration of Trial:** Two years **Expected starting date:** 1 August 2023 **Expected termination date:** 31 July 2025	Expected starting date to be determined by the NBMA
2	**Plant Information**		
	Toxicity and Allergenicity	Cowpeas are not a source of toxins; however, like other grain legumes, cowpeas naturally contain a range of anti-nutritional factors such as phytic acid, hemagglutinin, tannin, trypsin inhibitors, oxalate, phytate, polyphenols, and oligosaccharides (OECD, 2018). Although allergic reactions to legumes, including peanuts and soybeans, are relatively common, allergy to cowpeas is rare. There is a single documented case where the analysis of sera from six individual patients exhibiting allergic reaction to cowpeas identified two proteins of 41 kDa and 55 kDa, respectively, as the major allergens of cowpea (Rao, 2000).	
	Describe the Intended Phenotypic Changes to the Plant.	The cowpea event described herein is intended to express the bean (*P. vulgaris*) alpha-amylase inhibitor-1 (αAI-1) protein in the seed as a means of controlling postharvest seed damage due to the bruchid *Callosobruchus maculatus*, known as cowpea weevil. There are no other intended changes to the plant phenotype.	
	Intended Reproductive Effects	The genetic modifications resulting in the cowpea event described herein were not intended to alter the reproductive or survival biology of cowpea. The standard conditions for ensuring reproductive	

		isolation of cowpea apply to the genetically modified cowpeas proposed for evaluation in the CFT.	
	What is the source of genetic material? Is the source of genetic material likely to affect the safe conduct of a Confined Field Trial? If yes, how?	<u>*Phaseolus vulgaris* L. (common bean):</u> The common bean, *P. vulgaris*, was the source of the *αAI-1* gene and its regulatory sequences, the leucoagglutinating phytohemagglutinin (PHA-L; *dlec2*) promoter and termination sequences. Common bean is a major grain legume that is consumed worldwide for its edible seeds and pods (OECD, 2015b). According to the Consultative Group on International Agricultural Research (CGIAR), common bean provides protein, complex carbohydrates, and valuable micronutrients for more than 300 million people in the tropics. In many areas, beans are the second most important source of calories after maize. In spite of good nutritional quality, common beans contain some constituents having anti-nutritional effects, such as tannins, phytates, protease inhibitors, and lectins. <u>*Streptomyces hygroscopicus:*</u> The *bar* gene, which confers resistance to the herbicide bialaphos (glufosinate ammonium), was introduced to allow positive selection of transformed plants during tissue culture regeneration and was originally cloned from the bacterium *S. hygroscopicus*. This bacterial species is a saprophytic, soil-borne microbe that is not considered a pathogen of plants, humans, or other animals (Locci, 1989). None of the donor organisms used as sources of introduced genes will affect the safe conduct of the CFT.	
	Changes in Toxicity and Plant Composition	The genetic modifications resulting in the cowpea event described herein were not intended to result in any changes to the potential toxicity or allergenicity of cowpea, nor any significant changes in the composition of derived grain or forage.	

	Describe the features of the genetic construct?	*Agrobacterium*-mediated transformation was performed essentially as described by Popelka *et al*. (2006) using plasmid pPOP3.3 which contains two gene expression cassettes on separate T-DNA segments. The use of two T-DNA segments, one containing the *bar* gene expression cassette and the other containing the *αAI-1* gene expression cassette, allows for removal of the herbicide-tolerance selectable marker by genetic segregation during breeding.	For details on the description of the genetic construct, refer to application dossier (pages 8-10)
3	**Trial Description**		
	Experimental Design	The purpose of the field trial is to evaluate 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 trial will also be used to produce seed (grain) for postharvest storage assessment of resistance to bruchid damage and for compositional analysis of key nutrients. Five entries will be planted at each field trial site, one test entry (event CSI32) expressing the αAI-1 protein for control of bruchid infestation and four control entries. The experimental design is a randomized complete block design (RCBD) with six (6) blocks (replicates) of each entry. Each plot will be 2.25 m × 5 m and will be planted with four (4) rows of 26 plants, with an inter-row spacing of 0.75 m and a within-row plant spacing of 0.20 m, for a total of 104 plants per plot and a planting density of 67,000 plants per hectare. The spacing between adjacent plots within a block will be 1 m and the spacing between adjacent blocks will be 1.5 m. The dimensions of the entire trial site, excluding the isolation distance, will be	See Table 3 and figure 2 of the application dossier (pages 12-13)

		15.25 m × 37.5 m for a total area of 571.9 m^2 (Figure 2). The trial site will be located at least 30 m from any other cowpeas undergoing trial within the CFT facility to avoid any effects of pesticide drift and potential out-crossing.	
	Are there wild plant species in the vicinity of the trial site that could be fertilized by pollen from the trial plants, resulting to viable seeds?	There are no plants of wild species near the trial site that could be fertilized by cowpea plants in the trial. Recent scouting trips around the IAR farm showed no presence of wild cowpeas. The nearest point where these species were spotted was more than 800m away from the CFT site, thus the possibility of outcrossing from cowpeas in the trial to wild cowpeas is near zero.	
	Describe mechanisms in place to prevent pollen-mediated gene flow from the plants in the trial sites.	In consideration of outcrossing data for cowpeas reported by Fatokun & Ng (2007), an isolation distance of 30 meters is sufficient to mitigate pollen-mediated gene flow from cowpea plants within the CFT facility to other sexually compatible species. The isolation zone will be contiguous around all four sides of the CFT site and will be monitored at least once every four (4) weeks during the growing season for the presence of any prohibited plant (e.g., cultivated or wild cowpea). The monitoring activity will be recorded in the Record of Current Season Monitoring and any prohibited plants identified during monitoring will be destroyed before flowering by uprooting or spraying with a broad-spectrum herbicide (e.g., glyphosate) so they do not flower and set seed.	
	Describe measures in place to control trial plant volunteers after termination of the trial.	As previously noted, cowpea cultivars are generally not capable of creating long-lived seed banks in the soil because their seeds are permeable to water and lack dormancy (Lush et al., 1980). Cowpea seeds typically germinate and emerge from the soil within 7–10 days, thus we propose that five weeks of postharvest monitoring will be sufficient. Upon completion of the trial,	

		the site will be put under irrigation to induce germination of all cowpea seeds that may have become soil incorporated. All volunteer plants will be uprooted prior to flowering, collected into secure bags, and destroyed on the site by deep burial or burning.	
5.	**Material confinement**		
	Packaging	Seeds of the regulated genetically engineered cowpeas will be packaged in tough waterproof material carried in a plastic container with a tight-fitting screw-top lid. The plastic container will be labelled clearly with a matching label inside. The consignment will be accompanied by the requisite phytosanitary documentation and will be received at port of entry in Nigeria from Canberra, Australia, by both IAR and Plant Quarantine Services staff. The material will be transported by road to IAR laboratories in Zaria under the supervision of the Principal Investigator for secure storage pending start of the proposed trial. Following planting, all remaining unplanted seed will be returned to the secured seed storage locker. Packing containers and materials will be sanitized free of residual plant material or destroyed. All records, including the import permit, Record of Shipment and Receipt, and records of inspection, will be maintained in the Compliance Document Binder at IAR CFT site, with copies transmitted to the DDPSC for archival.	
	Harvesting, Transport and Storage	At crop maturity, pods will be hand-harvested into clearly labelled bags and transported to the nearby IAR laboratory for threshing and data recording. Harvested seeds from each field trial entry will be subjected to a storage stability assessment at IAR to measure resistance to damage from bruchids and a portion of	

		each harvested seed lot will be reserved for nutrient composition analysis. A Record of Storage Inspection and Inventory will be maintained for all retained regulated plant material and these records will be made available for inspection by NBMA officials upon request.	
	Disposal and Clean-up	All equipment and utensils used on the trial site shall be cleaned by washing at the trial site to eliminate unintended movement of regulated plant material from the site. Any residual plant material recovered from equipment or utensil washing, or from other field trial activities (e.g., planting, monitoring, rogueing, and harvesting) that is not approved for retention and transport to the laboratory will be destroyed on the trial site by burning or deep burial and this activity will be supervised and recorded in the Compliance Document Binder by the Trial Manager.	
	Site Security	The proposed CFT site is secured with a 6-ft high chain-link wire fence to keep out unauthorized personnel and/or livestock. Appropriate signage is placed on the fence indicating that entry by unauthorized personnel is prohibited. The site has a lockable gate to allow access by authorized personnel only and a logbook is maintained to keep a record of the names, purpose, dates, and times of all entries to the site. During the trial, the site will have a 24-hour round the clock security patrols by IAR personnel for the sole purpose of enforcing security at the trial site. The security staff will have undergone a training programme on Trial Site Management alongside other project staff. This site has been accredited and periodically reviewed by NBMA since 2009.	Convert 6ft to metres for consistency.
6.	**Records, Personnel and Planning**		
	Other reports	All records will be placed in a Compliance Document Binder maintained at the IAR	

		CFT facility and will be always available for review by inspectors designated by the NBMA. Duplicate copies of all records shall also be transferred to the DDPSC for archival. The compliance forms to be completed and maintained will include • <u>Record of Shipment and Receipt</u>**:** To document movement of regulated plant material to IAR, Zaria, from CSIRO, Canberra. This form is also used to document the movement of harvested plant materials from the trial site to the IAR laboratory after harvest. • <u>Record of Planting:</u> To document the exact number of transgenic cowpea seeds of each modified line planted in soil in the CFT site, the exact number of seed not planted, and the sterilization and /or destruction of any shipping containers. • <u>Record of Current Season Monitoring:</u> To document the monthly monitoring of the 30 m isolation distance as well as to document the devitalization of any prohibited plants found to be within the isolation area. • <u>Fertilizer and Pesticide Usage Form:</u> To document all fertilizer and pesticide applications before, during, and after harvest of the CFT.	

		• Record of Corrective Action: In case of any breach of reproductive isolation, the NBMA will be immediately notified by phone of the incident and a record of corrective action will be completed. • Record of Harvest/Destruction: To document the harvest and/or destruction of the trial. • Record of Post-Harvest Monitoring: To document the weekly post-harvest inspection of the post-harvest site for living cowpea plants and destruction of volunteers. • Record of Storage Inspection and Inventory: To document all additions and removals of regulated genetically engineered plant material in storage at IAR, including inspections of the integrity of the storage facility. The data forms to be completed and maintained will include: • Growth & Development Bi-Weekly Rating Form: Plant stand, growth stage, vigour, plant height, and qualitative assessment of normal growth will be collected at least every two weeks. Stand emergence (germination) and days to first flowering and days to 50% flowering will also be recorded. • Pest & Disease Bi-weekly Rating Form: Observations on cowpea diseases (e.g., Anthracnose, Septoria, Cercospora, Rhizoctonia stem rot, Sclerotinia rot, Fusarium wilt, mosaic virus, yellow mosaic virus, brown leafspot, white leafspot) and pests (e.g., aphids, thrips, *Maruca*, pod-sucking bugs, blister beetles, leafminers,	For Harvest Data Form, see Table 4 (Page 13)

		millipedes, *Spodoptera*, whiteflies) will be recorded every two weeks during the growing season. • Harvest Data Form: At harvest maturity (growth stage R8), data will be recorded for each plot for each of the relevant parameters. • Meteorological Data Form: Rainfall, relative humidity and temperature will be recorded daily. **Personnel:** Scientists and technical staff at IAR have carried out several CFTs of genetically engineered cowpeas since 2009. As a refresher, prior to planting, all trial personnel will undergo a training course at IAR, Zaria, on current and postharvest compliance management, including a review of all relevant Standard Operating Procedures (SOPs), compliance record keeping requirements, and work instructions for data collection and recording. This training will be recorded in the Compliance Document Binder that will be maintained at the site for the duration of the CFT.	
	Contingency Plans	In the highly unlikely event of an accidental release of regulated plant material, the Principal Investigator, the IAR Institutional Biosafety Committee (IBC), and the NBMA will be notified immediately by telephone and in writing within 24 hours. The accidental release of regulated plant material shall be immediately documented in a Record of Corrective Action. The Trial Manager shall retain the original record in the Compliance Document Binder, and copies shall be immediately submitted by facsimile to the Principal Investigator, the IAR-IBC, and the NBMA. If an accidental release affects an area outside the perimeter of the trial site, that location shall be marked, monitored, and shall be treated in the same manner as the	Information on contingency plan is adequate

		trial site with respect to ensuring that no additional release of material occurs. Consultation with the NBMA will determine the period of monitoring required and the most appropriate course of action to mitigate any spread of regulated plant material following accidental release. In the unlikely event of unauthorized access to the trial site or a natural disaster that affects the integrity of the CFT, the IAR-IBC, and the NBMA shall be notified immediately, and any remedial action shall be determined in consultation with the NBMA.	
	Recovery of materials	In addition to the security measures and contingency plans put in place, measures for the recovery of materials that may be inadvertently lost should be put in place.	

RECOMMENDATION

1. The Curriculum Vitae of the Principal Investigator and the Trial Manager should be provided.
2. The applicant should be consistent in the units of measurement.
3. Expected date of commencement and termination should be determined by NBMA after approval.
4. All relevant regulatory agencies (Agricultural (plant) Quarantine, Customs, Seed Council etc.) concerned with material transfer should be involved.
5. The NBC having gone through the document submitted by the applicant and the assessment and recommendation by the NBTS, hereby recommend to the NBMA to approve the application subject to correction of all the observations.

NBC Members

s/n	NAME	ORGANISATION	SIGNATURE	DATE
1	Prof Celestine Aguoru (Chairman)	Federal University of Agriculture, Makurdi		
	Dr. Rose Gidado	NABDA		
2.	Dr. Gabriel Malomo	ARCN		
3	Mr. Benson Ajibi	NAQS		
4	Mr. Samuel Gbenga Sulola	NCS		
5	Barr. Habiba Chime	FMoJ		
6	Dr. Nkechi Mba	FCCPC		
7	Prof. P.O Anyaegbu	UNIABUJA		
8	Mr. Emmanuel Ude	RMRDC		