Bt Cowpea Field Trial Planning Meeting Report

Donald Danforth Plant Science Center, St Louis, Missouri

17-18 December 2008

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Acknowledgements

The African Agricultural Technological Foundation (AATF) wishes to acknowledge the support accorded to the *Bt* cowpea project by the various stakeholders. Special thanks to TJ and his team for the successful completion of the Puerto Rico trials. AATF also thanks the USAID for providing the much needed funds for the project. Lastly, thanks to the various professionals and researchers who have shared their experience and offered expertise during the initial setting up of the project. AATF is indebted to your efforts and look forward to your continued support.

Foreword

The *Bt* cowpea field trial planning meeting was held on 17–18 December 2008 in Donald Danforth Plant Science Center, St. Louis, Missouri. The two day meeting was organised jointly by the African Agricultural Technological Foundation (AATF) and the Program for Biosafety Systems (PBS) bringing together the project partners, and leading cowpea scientists (PIs) from the three pilot countries and from Puerto Rico (PR). The meeting came soon after a planning and review meeting held on 2–4 June 2008 in Abuja and a Project Advisory Committee (PAC) held on the 9–10 February 2008 in Washington DC. The PAC meeting discussed plans for the first ever Confined Field Trials (CFTs) of *Bt* cowpea while the Abuja review meeting outlined the milestones for the *Bt* cowpea project activities. The meetings recognised the efforts made by a number of people to acquire the *Bt Cry1Ab* gene and to develop efficient transformation systems to introduce the *Bt* gene into cowpea at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia. It was therefore critical to get the plants into the field and get the real estimate of whether the first batch of developed lines could provide sufficient levels of resistance to *Maruca*. The first CFTs in PR whose results are discussed in this report are therefore a critical milestone in the development of *Bt* cowpea. These first trials have yielded important results and there is need to embrace these first successes and move the test to the next stages. Generally, this report outlines a review of the CFTs in PR and recommends suggestions for improvement in the subsequent trials in PR and west Africa. The report also outlines the road map for developing CFTs in Africa and the training needs for the different National Agricultural Research Systems (NARS) partners.

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PBS

Acronyms and Abbreviations

AATF	African Agricultural Technology Foundation
BF	Burkina Faso
Bt	*Bacillus thuringiensis*
CFTs	Confined Field Trials
CSIR	Council for Scientific and Industrial Research
CSIRO	Commonwealth Scientific and Industrial Research Organization
ELISA	Enzyme-Linked Immunosorbent Assay
GMO	Genetically modified organism
IAR	Institute of Agricultural Research
IBC	International Biosafety Committee
IITA	International Institute of Tropical Agriculture
INERA	Institut del'Environnement et de Recherches Agricoles
LI	Legal Instrument
NAB	National Agency for Biosafety
NARS	National Agricultural Research Systems
NBC	National Biosafety Committee
NGOs	Non-Governmental Organisations
PI	Principal Investigator
PBS	Program for Biosafety Systems
PR	Puerto Rico
SARI	Savannah Agriculture Research Institute
USAID	United States Agency for International Development

Executive Summary

The cowpea pod borer (*Maruca vitrata*) has persistently affected the yield of cowpea (*Vigna unguiculata*) in most west African countries (Murdock et al 2007). There is need to acquire a more naturally resistant variety. AATF is currently facilitating a process of developing a new genetically modified cowpea with *Bt* gene that would enable resource-poor farmers in Nigeria, Ghana and Burkina Faso have access to high quality seed and socially acceptable cowpea varieties with increased resistance to *Maruca*. To achieve this goal, a Cowpea Project Review and Planning Meeting was held in Abuja, Nigeria in 2–4 June 2008. The meeting led to the development of a comprehensive work plan of activities, project milestones and timelines up to 2014, when the *Maruca*-resistant cowpea varieties are envisaged to be available for adoption by farmers. As one of the milestones in product development, the first Confined Field Trials (CFTs) on *Bt* cowpea was initiated in August 2008 in Puerto Rico (PR) to test efficacy against the pod borer. This initial field trial, led by TJ Higgins, Jeff Stein and Larry Murdock was designed to assess the first set of *Bt* cowpea transformation events created by TJ Higgins at CSIRO. A team representing the project partners and potential donors visited the trial site in Puerto Rico between 6–8 October 2008 to evaluate the performance of the events against the pod borer in the field. Although preliminary, the team together with Dimuth Siritunga and Fernando Covas of the University of Puerto Rico – Mayaguez, concluded that the trials were promising and could generate useful data on resistance, yield comparisons and issues of gene flow. The findings of this trial along with those of the bioassay of *Maruca* on cowpea flowers were discussed in this review and planning meeting and are reported in this proceedings.

Against this background, the two-day review and planning meeting specifically sought to:

a.) review the Confined Field Trial (CFT) work plan established in Abuja in June 2008, and to update on the status of CFT applications and training needs of project partners in Nigeria, Ghana, and Burkina Faso

b.) review the methodology used in the first trial in Puerto Rico and evaluate the insect efficacy data and agronomic observations from the first trial

c.) review progress on the molecular and genetic characterisation of transformation events for the 2008 field trial and identify candidate events for the 2009 field trial

d.) formulate a refined trial design and protocol for the anticipated second trial.

The meeting's outputs were:

1) progress towards the introduction and testing of *Bt* cowpea in Africa was established and training needs of project partners in CFT management were identified
2) the insect efficacy data and agronomic observations from the first trial in Puerto Rico were evaluated
3) candidate events for future efficacy trials were selected
4) field trial design and methodology for CFTs was discussed and standardised.

Participants
1st row: (left – right) Ibrahim Atokple, Prince Addae, Dimuth Siritunga, Venu Margam, Nompumelelo Obokoh, Clementine Binso, Jeff Stein, Jeff Ehlers
2nd row: (left – right) Francis Nang'ayo, Fernando Covas, Jacob Mignouna, Mohammad Ishiyaku, Larry Murdock, Joe Huesing, TJ Higgins

Review of the Confined Field Trial Work Plan Established in Abuja in June 2008

Background

A comprehensive plan of action to guide activities leading to the development and deployment of *Bt* cowpea was developed during the meeting held in June 2008 at Abuja. AATF has been working with partner countries on the implementation of the plan of action. This plan outlined the activities essential for obtaining regulatory approval (Quarter 4, 2008), as highlighted in the *Maruca* resistant cowpea project work plan, and includes the application of CFT permits, identification of principle investigators (PIs) at different pilot countries, and identification of training needs of the different project participants.

1.0 Status of CFT applications in the NARS pilot countries

1.1.1 Status of CFT applications in Nigeria

Currently, Nigeria does not have a biosafety law in place but there is a biosafety guideline approved by the Federal Executive Council that outlines the mode of operations for research purposes but not for commercial utilisation of biotechnology-derived products. There is a National Biosafety Committee (NBC) that consists of a chairman, secretary resident in the Biosafety unit in the Federal Ministry of Environment and Civil and Non-Civil Representatives. The NBC has powers to decide on the procedures of product development and in confined field trials. If there is enough evidence that there has been enough research on the product or if a similar product has been developed elsewhere, the NBC may authorise the release of the product for consumption.

The cowpea CFT application was filled and submitted for assessment and processing in July 2008. The Institutional Biosafety Committee (IBC) at IAR, Zaria was formally inaugurated on 18 July 2008. The IBC met in October 2008 to review the CFT application and it was approved with minor modifications. The application was subsequently submitted to the National Biosafety Committee (NBC) and will be assessed by the sub-technical subcommittee. The Principal Investigator, Mohammed Ishiyaku, is in constant contact with the biosafety officers and a good rapport has been established. The trial site has been identified and fenced off and AATF has committed funds for these activities including the processing of the application.

1.1.2 Status of CFT applications in Burkina Faso

Burkina Faso is one of the countries with a fully functional biosafety framework. In 2004, a biosafety framework was outlined and in 2005, a National Agency for Biosafety (NAB) was launched. The bill was passed into law in 2006. In 2008, after several CFTs on *Bt* cotton, the process was moved forward to increasing seed for commercialisation of *Bt* cotton. The *Bt* cotton project has set a good precedence for *Bt* cowpea.

It was established during the planning meeting in Abuja that the National Agency for Biosafety (NAB), meets only once per year (in June). INERA would help in filling and submission of the form as well as in identifying the appropriate testing site. INERA had by August 2008, identified two possible *Maruca* hot spot sites for the CFTs but a field survey of the sites was required to decide on the most appropriate field for the CFTs. AATF would provide funds for the application process and for the field preparation. The application will be submitted in June 2009. The ANB which has a new chairman would meet in late December 2008 to review the form. The Principal Investigator (PI), Mme Clementine Dabire, will follow up on the application form.

1.1.3 Status of CFT applications in Ghana

AATF held discussions with the Secretary of the NBC who clarified that Ghana's Biosafety Bill has since October 2004 not been passed into law. To circumvent the delay in the passage of the Bill and allow for the practice of safe science, a biosafety Legal Instrument (LI) was promulgated, presented before parliament and passed. The LI uses the existing CSIR Act 521 of 1996 as a framework law since it has provisions for the conduct of research in general. The NBC members and inspectors from regulatory agencies have been trained on inspection for compliance. Submission of an application for CFT to the NBC does not attract any fees. Ghana therefore looks forward to reviewing the application and conducting the planned CFT on *Bt* cowpea.

The CFT application form is almost complete. The meeting recognised the need to form a small team to address the remaining parts of the CFT application. Funds towards the development of the trial field have been disbursed by AATF. Efforts should be made to have the application submitted to the IBC by January 2009. In addition, the following steps were recommended: (i) The PI, Atokple, to inform the former Director General of SARI (Dr Salifu), who is already aware of the forthcoming CFT activities in Ghana, to help push the application forward; (ii) discuss with the partner institution (SARI), details of experimental design and confinement for application; and (iii) determine additional needs at trial location.

1.2. Training needs for anticipated field trials in Africa

The meeting identified weaknesses in the African countries that would hinder their optimal participation in the development and deployment of *Bt* cowpea. Many of the observed gaps related to training on the various aspects for the project execution and for the ultimate deployment and utilisation of the *Bt* cowpea. Professional training is critical for undertaking research. Generally, the meeting noted that training was required for three main teams that would be responsible for the successful transformation and adoption of *Bt* cowpea.

1.2.1 The investigative team

This includes the people involved in the daily management of the cowpea CFTs from planning to deployment. These consist of the scientists including, the PIs, the entomologists, breeders and technicians. The meeting was informed that there are several scientists trained on biotechnology in west Africa but they still require training in order to undertake *Bt* cowpea work. It was noted that specialised training was required to equip the team with modern technologies of plant biotechnology. It was agreed that it would be economical to hold joint training sessions in one of the west African countries. To this effect, the meeting agreed to source for appropriate facilitators for the training programme. It was also agreed that appropriate short courses on biotechnology issues relevant to *Bt* cowpea would be held from time to time.

1.2.2 The regulatory team

This includes the bodies that offer the legal advice and regulates the affairs of the project such as the IBCs, NBCs and other decision makers, mandated with approving the conduct of the projects. The meeting was informed that some countries such as Nigeria and Burkina Faso had NBCs and IBCs already in place but it was noted that there was need for more training to improve on their efficiency in reviewing and evaluation of CFT applications. It was agreed that field visits to Puerto Rico would go a long way in changing the perception of the regulators with regard to GMOs. There is need to extend invitations to the regulatory body nominees to the cowpea meetings. Sharing of experiences on other GMOs that have been developed will aid in improving the perception of the products.

1.2.3 Others

This team consists of the wider community including the media, civil and non civil groups, NGOs and farmers. This group is very important since it would play a crucial role in the product deployment and its ultimate adoption. The group in particular will play a key role towards public acceptance of the *Bt* cowpea. The meeting was informed that there were

various concerns raised by some groups on the adoption of the *Bt* cowpea. These include the following.

1. *Effects on health and environment*: The meeting noted that this concern was premature for now since the product is still at the development stage. The members were however assured that the project would adhere to the food and feed biosafety policy guidelines of the partner countries, thus there will be no commercialisation of the product until the respective countries approve it.

2. *Loss of local varieties*: Issues were raised over the possible impacts the *Bt* cowpea could have on the local varieties. Some countries raised concerns that with the adoption of the *Bt* cowpea, farmers may completely neglect the local varieties leading to their total elimination possibly leading to loss of national heritage. The meeting clarified that only farmers willing to plant the *Bt* cowpea will do so. Training is therefore needed to ensure farmers make informed decisions. The meeting also noted that the respective countries had germplasm conservation programs in place for preserving their elite varieties.

3. *Lack of information on GMOs*: It was noted that many potential end users of *Bt* cowpea are illiterate and have little or no knowledge on the GMOs. It was established that even the people who have ideas on GMOs did not know the difference between breeding and genetic transformations. The meeting agreed that there was need to have farmer field schools on GMOs. It was also deemed necessary to work closely with institutions such as universities, colleges, NGOs, media houses and other lobby groups to ensure a wide coverage of the targeted groups.

1.3 Issues of discussion and way forward

BioCassava Plus project experience in Nigeria: The Product Development Manager for the BioCassava Plus project, Martin, shared his experience of CFT applications in Nigeria. To help steer the process of setting up the CFTs of *Bt* cassava in Nigeria, the cassava project appointed a former national biosafety official (Matthew Doore) as a consultant, to help fill the application and secure approval. The application for the *Bt* cassava CFT was submitted on 27 November 2008. The NBC met on 16 December 2008 and appointed a technical subcommittee of three to visit the field area, review the application and submit a report to the NBC by end of January 2009. Once the NBC presents the report to the Ministry of Environment for approval, there may be need for ministerial lobbying to hasten the approval. A fee of US$ 500 was paid for the processing of the application and US$ 5,000 was paid to have the NBC meet. Other expenses required include: payments to IBC members, accommodation and travel expenses for the subcommittee members.

Harmonisation of the *Bt* cowpea and the BioCassava Plus projects' CFT application activities: The meeting recognised the need for the two projects to synchronise their efforts in order to get the approval from the Ministry of Environment.

Import permits: To achieve the milestones to have CFTs in the west African countries, there should be a consideration for training and a need to have permits from quarantine offices for the importation of *Bt* cowpea seed.

CFT application expenses: AATF will facilitate the financing of the application processes and other regulatory activities towards the establishment of the CFTs.

Training needs: It was resolved that for NARS countries, all staff that will be involved in the CFT will be trained on pertinent issues of material and genetic confinement to facilitate compliance with in-country regulations governing confinement of GM crops. The meeting resolved that there should be a training course on CFT site management targeting personnel that will be involved in the conduct of CFTs in Burkina Faso, Ghana and Nigeria prior to commencement of the trials. AATF and PBS plan to hold training on CFT site management for staff of NARS partners during the first week of June 2009.

Review of *Bt* Cowpea Confined Field Trials in Puerto Rico

Background

Out of the six experimental stations in Puerto Rico (Isabela, Adjuntas, Corozal, Lajas, Luana Diaz and Gurabo), Adjuntas was chosen for the *Bt* cowpea trial since it is a hot spot for the *Maruca* pest feeding on white beans commonly grown by smallholder farmers. To launch the CFTs in PR, three levels of permissions were obtained: from the United States Department of Agriculture's Animal Health Inspection Service and Plant Health Inspection Service Biotechnology Regulatory Service (USDA–APHIS BRS), the Puerto Rico Department of Agriculture, and the Institutional Biosafety Committee of the University of Puerto Rico, Mayaguez. The CFT was approved in March 2008 following an application by the project partners to import 15 transgenic cowpea lines containing the *cry1Ab* gene, which confers resistance to *Maruca* by cowpea. The seeds were received in mid-June and preparatory planting started immediately (17 June – 12 August). The delegate of project partners consisting of TJ, Jacob Mignouna, Mohammed Ishiyaku and Larry Beach, visited the trial for evaluation. Four days after the partners' delegation, on 12 October, the field was inspected by the USDA BRS officials. Harvesting of the transgenic lines was done on the 28 October and the field was left idle for six months post-harvest evaluation.

2.1 Experimental design

A split plot experimental design was adopted that included white beans that lined the four corners of the field, three border rows of a local variety, IT86D 1010, planted one week apart forming the outer border row, middle border row, and a final border row. The inner rows of the experiment comprised of one rep of the non-segregants and finally the three reps of the transgenic lines. The negative segregant was included in the trial to determine if there was a tissue culture effect since the isoline will have undergone all the processes except the insertion of the transgene. Twenty seeds were planted per row at a distance of 20cm apart. A combination of rain and hurricane destroyed one rep of the transgenic lines. To ensure there was enough pressure, *Maruca* were released twice. In the first release, *Maruca* pupae were collected in a container and left in the field at strategic points to ensure they infested the plants. For the subsequent release, 344 *Maruca* moths were mated then released to the field in the late afternoon.

2.2 Analysis of data from the Puerto Rico CFT

Data from the transgenic plants was collected based on plant vigour, pod yield, pod damage and pod weight/plant and were subjected to analysis.

2.2.1 Agronomic analysis

Phenotypically, the transgenic plants and their non transgenic segregants were vigorous and had the same phenotype as the wild type except for one line (Tg 705C1) that showed stunted growth. On average pod yield was low (50%–60%) in the transgenic lines compared to the controls (non segregants and IT86D). However, a very high standard deviation for pod yield was obtained for the negative segregants. This was attributed to the fact that only one rep of non segregant versus three reps for the transgenic lines was used. Similarly, the transgenic lines had reduced pod weight/plant compared to the parent line and the non-segregating lines but there was no major difference in the number of pods per plant.

2.2.2 *Cry1Ab* expression during the trial

The plants were assayed for expression of the *Bt* gene at the leaf stage. A quick *Bt* strip test was used to confirm the expression of the *Cry1Ab* protein in the transgenic lines. Briefly, three leaf discs are placed in an appendorf tube containing buffer and the *Bt* strip is slowly lowered into the buffer. Formation of a single line proves that the test is working. Appearance of a second lower line shows *Cry1Ab/Ac* protein is present (Figure 2.1). It was established that all the transgenics had the *Bt* protein except one line (705A).

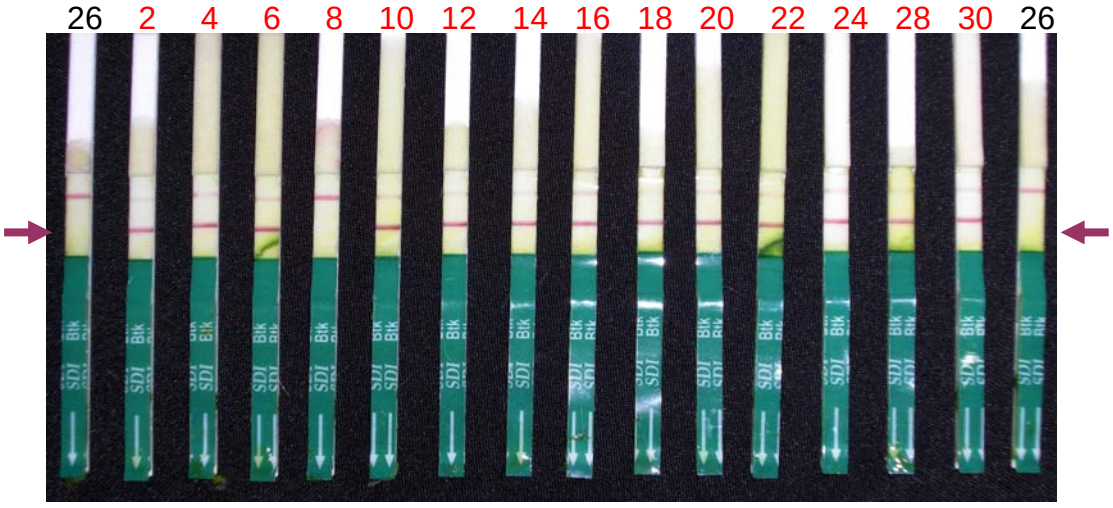

Figure 2.1: The qualitative *Bt* test strip showing the presence of the *Cry1Ab* protein in 13 transgenic lines (lane 2-30, except lane 8) and no expression in the wild type control, IT86D-1010 (lane 26).

2.2.3 Insect efficacy

Generally there was a positive effect of the transgene on efficacy. The pod damage on the local PR cowpea was quite high compared to the transgenic plants. *Maruca* seemed to prefer the local variety probably due to its large size, more flowers and open canopy. The rainfall may have reduced the infestation level, since before the rains the pod damage was 40%–60%. Infestation was poor after the rains an indication that the insects may have drowned. There was also a hurricane prior to the flowering season which may also have affected the infestation. Probably in the absence of these poor conditions, the infestation would be improved. It is therefore important not to draw conclusions from these trials, especially, since only one rep was included for the negative segregant and the hurricane destroyed some of the transgenic lines.

(a) **(b)**

Figure 2.2: (a) Pod damage and (b) *Maruca* larvae feeding inside the green pods of the negative segregant

(a) **(b)**

Figure 2.3: Larvae found in flower on: (a) non-transgenic; and (b) transgenic plants

2.3 Issues of discussion and way forward

Trial design: The meeting noted that the design and the timing for the trial could have compromised the results obtained. There is need to come up with a design which could ensure maximum infestation was obtained. The meeting advised that all events in the trial should be planted at the same time to avoid biases due to differential planting and flowering times. A positive control should be included in the next trials to test the conditions. It was agreed that CFTs could continue using the lead events from the first trial but TJ should continue generating more events.

Sample size: There was concern over the choice of the sample numbers, it was agreed that a power analysis method will be employed while planning for the next trial.

Infestation pattern: The meeting noted that the different planting time of the rows may have influenced the pattern of the infestation since the local varieties came to flowering days before the transgenics. It was agreed that the bioassay tests will be performed for every line after which agronomical tests will be performed to ascertain the suitability of the lines for subsequent field trials. For comparison purposes, suggestions were made to do parallel green house trials to compare with the field trials.

Pod damage: The meeting noted that the extent of pod damage was not clearly defined in the first trial; this could have led to the low levels of pod damage recorded. It was agreed that pod damage would be considered if: a pod has clear penetration and exit hole with fecal particles, a pod has damaged seed, and if a pod has live *Maruca* larvae inside. A ranking system of the levels of pod damage should be used to score the pod damage. It was generally agreed that flower bioassays, pod damage and seed damage will be considered in identifying the lead events.

Molecular and Genetic Characterisation of the Candidate Transformation Events for the 2009 Trials

3.1 Current status of new *Bt* lines

In the candidate transformations events undertaken in 2008 in TJ Higgin's lab, 112 transgenic events have been generated out of which 24 events have been tested in the lab, and 17 were tested for backbone sequences. Five of the events (705BE-636643; 710B-648; CP147cotA2; 708A1 627; and 712F1 719) have no backbone sequences, no high levels for *Cry1Ab*, are homozygous and have single insertion. These five events are so far the promising events that will be included in the CFTs in Puerto Rico and in the west African countries. Leaf tests for breakdown products of the *Cry1Ab* protein found that the breakdown product concentrations increased with the age of the leaves. Tests to determine the levels of gene expression in different plant parts established that the *Bt* gene was present in the pollen, style and stigma. It was notably absent in the roots and immature cotyledon.

Table 3.1 The most advanced *Bt* cowpea lines being characterised for the next field tests

Line	Southern insert number	Backbone sequences	Homozygosity	Current generation (seed or plants)
703C-654	4	no		T2 seed
705BE-636643	1	no	Yes (confirmed in T2)	T2 seedlings
706AC-635650	2	no		T2 seed
710B-648	1	no	Yes (confirmed in T2)	T2 seedlings
CP147eaA1	5	no		T2 seed
CP147eaB1	1	yes		T2 seed
CP147eaC1	6	yes		T2 seed
CP147cotA2	1	no	Yes ? (confirmed in T2)	T2 seedlings
CP147cotB1	4	yes		T2 seed
651C1 591	1	yes		T2 seed
701B2 631	4	no		T2 seed
702F2 625	2	no		T2 seed
704G1 709	1	no		T2 seed
708A1 627	1	no	Yes (confirmed in T2)	T2 seedlings
709A3 675	1	no		T2 seed
711B2 665	1 (double?)	no		T2 seed
712F1 719	1	yes	Yes (confirmed in T2)	T2 seedlings
CP152B2	1			T2 seed
CP152C1	1			T2 seed
CP154C1	1			T2 seed
CP161B1	1			T3 seed
CP161D1	1			T2 seed
CP161E2	1			T2 seed
CP163 cot A1	4			T2 seed

3.2 *Maruca* bioassays in Canberra

After a long search for *Maruca* in Australia, they were finally discovered in 2008. There are currently some *Maruca* colonies being grown with the help of Susan Larry, an Australian entomologist, to increase their population for research work. These colonies will provide stock for infestations in the subsequent CFTs.

In a preliminary test, first insta *Maruca* larvae grown on artificial diet were used to infest two similar sets of cowpea lines planted a week apart (12/11/08 – 19/11/08 and 17/11/08 – 24/11/08), under similar controlled conditions in the green house. For both experiments, the average percentage of mortality was three times higher in the transgenics, and the average weight of surviving larvae was greatly reduced. Comparatively, the average weight of the surviving larvae found on the negative segregant (705C-*Cry1Ab* null) and the wild type, IT86D-1010, plants were substantively bigger in size, 33X and 20X, respectively. Since the expression vary over season, it will be important to determine the threshold levels that can control *Maruca* in the field.

Table 3.2 Two *Maruca* bioassays experiments tested on whole plants

Line	% Mortality*		Average weight of surviving larvae (mg)	
	Expt 1	Expt 2	Expt 1	Expt 2
IT86D-1010	21.5	8.2	32.8	27.1
705C-619-10 (Null)	4.5	9.1	55.2	43.7
705C-619-9	65.4	27.3	1.7	2.2
632E-480	59.2	36.4	2.27	1.9
710B-648	63.6	22.2	3.7	3.0

* Does not include missing larvae

3.3 Issues of discussion and way forward

Maruca bioassay: There was concern that the expression levels of the *Bt* gene could be low since there were survivors in the *Maruca* bioassay tests performed. There is need to fix all the variables to achieve 100% mortality.

Efficacy test: It was noted that the expression levels of the gene were determined based on leaf bioassays alone. The meeting recommended flower bioassays since this would give an idea of the concentrations that go into the pods. In addition, DNA tests can be undertaken for comparison purposes. Since the expression vary over time, the meeting noted that there is need to identify the threshold levels that can control *Maruca* and maintain that level for the other trials.

***Maruca* rearing and availability:** It was agreed that the project would facilitate a visit by Fernando to Manuele Tamo's laboratory to train on *Maruca* rearing. In addition, the NARS participants will be provided with artificial diets to start rearing of *Maruca* before the next CFTs in Africa.

More events: It was noted that finding a good event was rare so TJ was advised to continue generating more events until the lead event was identified. Seed from TJ will be available for planting in April 2009. This would include seed of the five positively tested lines and the other promising lines TJ would have produced.

Proposed Plan for the 2009 Field Trials

Proper planning is a key to any successful field trial. Based on the experience from the PR trials, there was need to carefully plan for the next trials in the west African countries. A plan was developed with the main goal of selecting leading events from the PR trials for breeding trials in the NARS countries. The criteria proposed for event selection will be according to the following order: i) molecular characterisation (single insert, homozygous and no backbone sequences); ii) efficacy testing (both in lab and field); iii) ELISA tests for quantitative analysis of gene expression; and iv) testing for stability and efficacy in African varieties after the introgression and how they perform during the in-country trials.

4.1 Experimental design

The plan proposed planting 5–10 events (the five new lines plus other promising lines from the PR trials), four reps of 20 plants each in rows of 4m long and a spacing of 1m between the rows and 20cm within the rows (this is good spacing to achieve good infestation since it would ensure *Maruca* larvae remain in the target plants). IT86D-1010, a local variety, will serve as a spreader. In this case there will be two transgenic lines surrounded by the spreader. The border and spreader rows will be planted at the same time. At least 900,000 IT86D-1010 seed will be required. It is necessary to order a higher number of seed (should order for about 16 lines as opposed to the 10 lines). Tests will be done at various stages to eliminate the plants which would not be carrying the transgene. Both flower and leaf bioassays will be conducted to determine the levels of the transgene. In addition to the field trials, a similar set of events will be developed in the greenhouse to generate parallel results to test the conditions. The greenhouse plants will also provide surplus seed for further trials since they will have been selfed. The plan recommends a field visit by a delegation from the project partners in the period between flowering and pod development to assess the trials and select promising lead events for inclusion in the African trials. At least trials should be undertaken in two different African countries to test on the validity of the events. To avoid biases resulting from genotype–environment interactions, backcrossing will be done to generate F1 progeny that can be used in the other Africa CFTs.

4.2 Data analysis

In generating data for the trial, several parameters would be considered including the stand count, pod damage, total number of pods per plant, number of pendicules per plant, flower infestations (early larval instars), and pod load (PL) yield index (seeds/plant), and 100 seed

weight for yield evaluation. Pod damage will be scored based on presence of larvae in the pods, clear hole on the pod-wall with faecal deposits, an exit hole with clear signs of webbing or by presence of seed with holes. All processes beginning from planting will be done systematically at the same time to avoid biases resulting from differential planting, flowering and infestation or harvesting.

4.3 New methodology of gene introgression

Jeff is the PI of the CGIAR generation challenge programme funded through the tropical legumes project to enable modern breeding of cowpea using new generation systems. The project started by developing cowpea genomic resources including cDNA, ESTs and a high throughput SNP genotyping platform for genetic and physical mapping, and for development of easy to use markers for major traits. The group has genotyped sets of cowpea populations and has so far about 1,300 markers on the genetic map. Out of the 1,300 markers, any two parents will have between 200–400+ polymorphic markers. This means there is a very nice set of micro-satellites that can be used for introgression work with the cowpea. This will give new opportunities to use marker assisted selection not only for individual trait by trait basis but also will act as stock for gene selection because there are so many markers from which to look for chromosome segments that can be used for backcrossing.

In the case of the *Bt* cowpea, the *Cry1Ab* gene is responsible for *Maruca* resistance. The *Bt* gene will be introduced to a local variety, using a marker associated with a chromosome segment, the F1 generation will then be backcrossed with a required local variety hence driving the gene much more effectively as opposed to the normal backcross. After every single backcross, the hybrid expressing the traits of the recurrent parent will be used for subsequent backcrosses. Backcrossing is expected to reduce the gene composition for the non recurrent parent by half thus several backcrosses are required to ensure the genotype of the recurrent parent is recovered. IT86D 1010 is a transformable parent and is used as the non recurrent parent to carry the *Bt* gene to the elite plant germplasms.

4.4 Issues of discussion and way forward

Planting time: It was agreed that December to mid August will be the best time to do the second CFTs in PR since mid-August to end of November is the hurricane season. The second trial will be conducted in March/April 2009.

Germination rate: The meeting noted that there was need to ensure a good germination rate was achieved. It was recommended that seed should first be sown in pitmoss before being transferred to the field. Some seedlings will be planted in pitpots to serve as reserves.

Negative segregant: Concerns were raised over the inclusion of the negative segregant in the current plan as they had not yielded any value in the previous trials. It was agreed that the negative segregants will not be included but instead, more lines would be characterised and included in the next trials.

Bioassays at Monsanto: There was a proposal to have Monsanto perform the bioassays. This is expected to cut down the costs. To this effect, AATF would contact Monsanto and request them for the service.

Efficacy scoring: The meeting agreed that efficacy scoring will be based on several parameters including flower infestations, pod load, pod damage and pod yield. Pod yield will be determined per plant. Pod damage will be scored at maturity of the pods.

Caging: There were concerns over the effect caging could have on the transgenic plants. Caging has been reported to affect the physiology of genes to express themselves that may affect the inherent agronomic potential of the plants. It was agreed that caging would be confined to only the greenhouse trials to ensure self pollination and to ensure enough *Maruca* manifestation per plant was achieved.

***Maruca* biotypes:** The meeting noted that there may exist several *Maruca* biotypes in the west African countries. To avoid biases that may result from such eventualities, backcrossing will be done to ensure F1 generations are produced that are more similar to the local varieties.

USDA application: To ensure the proper timeline for the CFTs is maintained, the meeting agreed that application for seed importation will be made by late January 2009.

Genotyping: Due to the sensitivity involved with backcrosses, it would be economical for the procedure to be outsourced from people actively involved in introgression. It was agreed that Jeff Ehlers would assist with the procedure. DNA or preserved plant tissues would be shipped to Jeff's laboratory. The cheapest procedure of introgression would be adopted.

Appendix 1: Training Needs for NARS Participants

Country	Team	Needs	Concerns	Comments
Nigeria	Investigative	• PI – Updated training on conducting transgenic breeding • Entomologist – Needs training on rearing and management of *Maruca*	• Loss of native varieties • Effects on health	• Insist on maintenance of germplasm storages • Confinement to keep off unauthorised people
	Regulatory	• IBC– specialised training to improve efficiency in processing, analysing and processing applications • NBC – General training (practical visit to PR) for exposure		Incorporate other scientists such as conservationists and plant breeders to win their confidence
	Others	• Sensitise general public on GMOs, media, civil and non-civil • Organise open forums for discussion on GMOs	Most end users are illiterate in relation to GMOs	
Burkina Faso	Investigative	PI, breeders and entomologists require training on CFT conducts, basic plant genetics and GMO principles	Most end users are illiterate in relation to GMOs	Include universities and other relevant groups to ensure a wide outreach
	Regulatory	Biosafety agents need field visits to PR including the heads of research centres, CEOs and other influential people	Lack of sustained, credible and thoughtful seed distribution	An ex-ante economic impact study for *Bt* cowpea to back up scientific arguments
	Others	Sensitise public on GMOs including activists and farmers	Opposition against GMOs by activists	
Ghana	Investigative	• PI is new in *Bt* project and needs training in aspects of *Bt* crops • The entomologists, pathologists, microbiologists and agronomists need to be equipped with modern genetic techniques	Most end users are illiterate in relation to GMOs	Ghana benefited from a USAID PBS training
	Regulatory	Several regulatory staff got some training on GMOs but they need some refresher courses		
	Others	Require a well developed communication network to reach all the people intended for the *Bt* cowpea including farmers, media, NGOs, civil and non-civil		

Appendix 2: List of Participants

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