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Final Project Report
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Pioneering development of a public-private partnership in the use of agri-biotechnology for sustainable solutions to insect problems on crucifer crops in India

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By

AVRDC-The World Vegetable Center

Title of the project

Pioneering development of a public-private partnership in the use of agri-biotechnology for sustainable solutions to insect problems on crucifer crops in India

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01 January 2006 – 31 December 2007

I. Executive summary

Diamondback moth (DBM), *Plutella xylostella*, is the most serious pest worldwide of all economically important Brassicas such as cabbage, cauliflower, broccoli, mustard, radish, and several leafy greens. Farmers prefer to use chemical pesticides for controlling this pest because these chemicals have an immediate knock down effect and are easily available in the local market when needed. Pesticides occupy a major share in the cost of cultivation accounting to 38% of production cost of major cole crops in parts of India. A significant portion of this cost is due to the spraying of inappropriate chemicals, excessive application, inappropriate timing, wrong combination of chemicals, and at times, spurious chemicals as a result of an increasingly important factor of the development of resistance to insecticides which leads farmers to spray even more pesticides. Development of insecticide resistance can occur within one or two cropping seasons immediately after the introduction of a new chemical. Insecticide resistance, environmental degradation, human health impacts, resource loss and economic concerns have triggered a growing interest in alternative safer management techniques such as *Bacillus thuringiensis* (Bt)-transgenic crops. The success of Bt-transgenic plants has already been demonstrated in crops like cotton, which has provided a 60-80% decrease in the use of foliar insecticides, amounting to about 15,000 tons of chemicals in China and 37 - 52% reduction in Australia. Though the evolution of resistance to the toxins produced by Bt plants remains a serious concern, recent experiments with a transgenic Brassica plant engineered with dual Bt-genes have shown to be far more resistant to the possibility of resistance development in targeted insects than single Bt gene. This should delay the possible development of resistance in DBM by several generations, which will provide an opportunity for developing a simple and more effective resistance management strategy.

Given the very high costs of developing and gaining approval for genetically modified (GM) solutions in agriculture and the need for this technology to be available to the poorest farmers, a public-private partnership (PPP) consortium namely *Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA)* was formed to address these problems. CIMBAA targets on the development and testing of dual gene system as part of a sustainable solution to Brassica pest problems and sets an example for biotech/seed industry on how to deal with GMO projects for orphan crops. The private partner takes the lead in developing the resistant plant material while public partners have an important role in testing the suitability of GM material for release and take the lead in developing an Integrated Pest Management (IPM) setting. The developed germplasm would be passed into public sector ownership (AVRDC) for dissemination and use by small-scale farmers. Engaging the public sector would lead to the creation of a genuine sense of ownership and allow the resistant transgenic plant material to be available for further breeding as a public

good. To understand the various elements and pioneer of this PPP, a project was funded by Vater und Sohn Eiselen-Stiftung, Ulm, Germany during 2006 and 2007. This project was implemented by AVRDC in collaboration with various Universities in Germany and India.

One of the major focuses in this project is to develop a legal strategy to transfer the ownership from the private partner (Nunhems) to the public partner (AVRDC), in order to ensure the availability of the dual-gene Bt brassica varieties to the poorest farmers in developing countries. The research collaboration agreement was signed between the partners to develop the dual-gene Bt brassica materials as well as to develop sustainable technologies to introduce the transgenic brassica with a full IPM setting. However, it was not so easy to develop the commercialization agreement between the partners, as we originally anticipated because of the liability issues which prevented the University partners to sign the agreement. Later, it has been decided to have a bilateral agreement between the private partner and AVRDC on the finished Bt vegetable brassica event(s) and their associated patents, which would enable AVRDC to make available the dual-gene Bt brassica varieties in the developing countries. However, University partners are now willing to participate in the commercialization agreement with suggested modifications, and the final agreement will be ready for signing in a few months.

Socioeconomic studies on production and protection of crucifers were conducted in three states *viz*., Gujarat, Karnataka and West Bengal in India based on a quantitative survey among 300 farmers including aspects such as attitudes on GM vegetables and pesticide use and costs. About 96% of the total respondents were using pesticides to control the pests on vegetable brassicas in India. Approximately 23% of the farmers applied a higher amount of pesticides than recommended. Farmers had sprayed pesticides as high as 248 applications in a cropping cycle. Nearly 65% of respondents did not use any protective measures while spraying, and about two-third of the total sample reported to have had some health related symptoms due to pesticide application. The awareness of farmers on GM crops was very low; only 2% of respondents were aware of GM vegetables and about 21% of respondents knew about other GM crops, especially cotton.

In addition, attitude of consumers on GM vegetables and pesticide residues was surveyed in five major cities in Karnataka, West Bengal and New Delhi. The urban households in India were generally well aware of the health risks associated with pesticide residues in foods. The mean of willingness-to-pay (WTP) for residue-free vegetables was more than 50% above current market prices. Among the households surveyed, 68% showed a positive attitude towards the introduction of Bt vegetables, while only 17% were mildly or strongly opposing the technology. Better education and greater exposure to mass media seem to reduce acceptance in the Indian context, partly reflecting biased information flows.

Furthermore, consumer trust in the public food safety authorities matters: those who consider the authorities ineffective were less willing to accept GM foods.

Diamondback moth, cabbage head caterpillar and cabbage web worm were susceptible to both Cry1B and Cry1C, which have been expressed in the dual-gene Bt brassicas. The imported cabbage worm was susceptible only to Cry1B and not to Cry1C. However, common army worm was not sensitive to both the toxins. Hence, this insect pest may pose problems in locations wherever it is epidemic on vegetable brassicas. The simulated field experiments with Xentari, a Bt formulation containing both Cry1A and Cry1C (as no formulation containing Cry1B and Cry1C is available) recorded low infestation of DBM, CHC and CWW. However, the sucking insects such as aphids and leaf feeding insects such as flea beetles were not controlled by Bt. Hence, dual-gene Bt brassicas should be introduced with a full-IPM setting to provide sustainable solutions for managing the pests on vegetable brassicas.

Bt did not have any significant adverse effects on the population of parasitoids like *Cotesia plutellae*, predators such as spiders and soil arthropods including coleopterans, dermapterans, hymenopterans, lepidopterans and arachnida. However, the population of ladybird beetles and field cricket was reduced in Bt treated plots compared to the untreated plots, although the Bt treated plots recorded higher numbers of these insects when compared to synthetic chemical pesticides. These results from the simulated field studies should be validated with actual dual-gene Bt brassica plants. The selection of elite events is being done in contained greenhouses and field trials in India by the private partner. When the elite events have been selected, they will be deployed for evaluating the impacts on non-target insects and natural enemies, with additional grants that will be generated for CIMBAA.

II. Activities and Milestone Achieved

<u>Objective 1</u>: Development of a legal framework of the transfer of patented technical know-how of dual gene Bt-transgenic crucifers from the private sector to the public sector as a pilot case for India

<u>Activities proposed in the proposal</u>

Bayer CropScience has developed the patented technical know-how of dual gene Bt-transgenic crucifers. Under satisfactory legal conditions the corporation would be willing to shift the ownership rights to the lead public sector institute, AVRDC – The World Vegetable Center, which in turn will forward the rights to concerned National Agricultural Research Systems (NARS) in India and from them to resident seed companies. Since this is the first attempt by AVRDC to be involved in the process of releasing transgenic varieties, we need support to explore the socially challenging and legally complex aspects of this process, including advise from legal experts on how to systematically shift the 'no fault' ownership rights to NARS of material approved for release by their national regulatory agencies.

<u>Milestones achieved in reporting period</u>

CIMBAA consists of one private partner (Numhems Seed Company, Bayer Crop Science) and four public partners *viz*., Natural Resources Institute, U.K; Cornell University, USA; Centre for Environmental Stress and Adaptation Research, Melbourne University, Australia; and AVRDC-The World Vegetable Center, Taiwan. Preliminary discussions and meetings that were held to define the Research Collaboration and Commercialization Agreements between the partners highlighted the numerous issues surrounding ownership and commercial release. Ideas were exchanged on whether the Research and Commercialization Agreements should be mutually exclusive. This would be done by either ensuring that there was no overlap between the two agreements *i.e*., the Commercialization Agreement would take effect only when the activities under the Research Agreement stand concluded; or alternatively a single agreement would be created that addressed both the Commercialization and the Research issues in detail. CIMBAA partners concluded that neither of these options was possible. As it was believed that addressing the numerous and complicated issues would take some considerable time to finalize, a decision was made (September 2005) to generate two agreements. The Research Agreement would cover the practical and experimental work which needed to initiate and include references to a Commercialization Agreement which would be developed later.

CIMBAA Research Agreement

As AVRDC has no legal representation in Taiwan, an external lawyer firm (Sean Butler, UK) was employed to study the agreement that was largely drafted by the private partner. The lawyer was asked to specifically represent AVRDC's best interest as a not-for-profit organization with the intent to eventually distribute the product for humanitarian purposes. The CIMBAA Research Agreement was drafted, distributed among the partners and signed in April 2006.

CIMBAA Commercialization Agreement

Bayer lawyers were initially encouraged to draft the Commercialization Agreement. It was then proposed that an Indian company be involved in the process to clarify Indian issues and present public side issues (Freedom to Operate (FTO), license transfer, liability, stewardship constraints under Indian Law). In April 2006, the lawyer office of Dr Malathi, in New Dehli, India was asked to help in the preparation of the CIMBAA Commercialization Agreement. Dr Malathi's office was also involved in the preparation of the agreement for Bt eggplant and so it was hoped that their recent involvement in that agreement would be directly applicable and help to streamline the process for the development of the CIMBAA Commercialization Agreement. Following discussions with CIMBAA project team members, the Indian Lawyers agreed to make a first outline. First draft produced was more of a list of concerns and issues and a request was made for a more detailed outline to be produced which incorporated the results of the discussions with the project team members.

Further meetings took place with Mr. Anil Mishra in New Delhi at the end of May, 2006 to discuss the progress of the CIMBAA Commercialization Agreement with particular reference to how to handle liability issues. The main change following this meeting was the proposal that the core vehicle for commercialization be a Special Purpose Vehicle (SPV) Company with at least Nunhems and AVRDC as shareholders. This company would hold the plant material and Intellectual Property Rights (IPR), and make applications to the Government of India. At registration, however, Nunhems withdrew leaving AVRDC as the sole shareholder. A framework for the proposed Commercialization Agreement was drafted as well as a framework for setting up a SPV in India. However, all the Universities (Melbourne, Greenwich (NRI) and Cornell) indicated that they would have problems being shareholders of an SPV. After further discussion the SPV was viewed as an additional unnecessary layer and so was dropped from the plans.

It was deemed necessary by AVRDC to have more frequent access to legal representation and so rather than paying professional charges to the lawyer for each interaction it was considered more cost effective to pay a lump sum and secure a lawyer for the next 18 months. A representative from Central Advisory Service (CAS) of the Consultative Group on International Agricultural Research (CGIAR) was selected. The CAS has a mission to serve the CGIAR Centers' needs regarding IP issues, by providing and facilitating expert advice and the exchange of intellectual property experiences among the Centers. Although AVRDC is not a member of CGIAR, it is a not-for-profit international organization with similar issues and so could benefit enormously from this relationship.

In 2007, we continued to work closely with CAS to develop the commercialization agreement. On March 24th, 2007 a meeting was held in New Delhi, India attended by representatives from Nunhems, AVRDC, NRI, and University of Melbourne to further work on the latest draft of the Commercialization Agreement. Following these discussions, CAS incorporated the decisions into a revised and streamlined draft. Further amendments were made by the public and private coordinators to the draft and then all CIMBAA partners were asked to seek their own legal opinion (from their own institute) on its contents in order to have constructive discussion at the Supervisory Board Meeting which was held in London on June 22, 2007. Several decisions were made at the London meeting in which representatives from all partners were in attendance. These decisions included the defining of “developing countries”, the Patent Cooperation Treaty (PCT) application to be made, and development of hybrids for final Bt product. A number of legal issues in connection with India were also identified by the partners and passed to CAS to consult with the Indian legal firm of Lakshmikumaran and Sridharan Associates who specializes in this type of work. It was identified that CIMBAA (as a non-legal entity) can be the holder of a patent in India. However, there were a number of subsequent issues as yet un-clarified, which may affect what structures are possible in the agreement.

Further to the outcome of the London meeting, all the University partners were concerned with clarifying the extent and limitations of any financial liability that they may have under this agreement (in maintaining and defending the patent but also in any arguments over IP use especially public liability for the eventually commercialized products). Moreover, lawyers from the University of Melbourne thought the current agreement was based on an inappropriate model and the agreement should be a simple license agreement wherein the legal entity that has the relevant rights to intellectual property (Nunhems) licenses those intellectual property rights to the legal entity that wants to commercialize/distribute the product (AVRDC). Following these discussions and the refusal of some partners to sign a commercialization agreement unless there was zero liability risk, a decision was made in October, 2007 to rewrite the agreement between only the relevant parties in this matter (Nunhems and AVRDC). The hope was that in dealing with only two parties the process would develop faster. However, there was still a move to have CIMBAA remains a group of five partners and further attempts have been made to keep all the universities on board when signing the actual commercialization agreement. The argument hinges on whether the commercialization agreement brings in any more or new liability over what is already there in the research agreement which was signed by all five partners. In the research agreement the intention of CIMBAA was to develop a GM-based solution for farmers in developing countries and all the partners were aware this would lead to the introduction of GMOs in the environment. The partners need to determine where the liability of the research agreement ends and where new liability of the commercialization agreement starts. At this time, mutually acceptable terms are still currently under review by the parties.

In developing the CIMBAA legal framework and maintaining close contact with CAS members, AVRDC has also been building capacity in the area of intellectual property.

A meeting opportunity for AVRDC and CAS members occurred in Rome, Italy in May 2007. A CIMBAA project team member was invited to attend the annual CGIAR IP managers meeting. At this meeting managers with expertise in intellectual property and technology transfer management met with a goal that participants would learn from each other and from additional, seasoned professional practitioners.

A CIMBAA project member (Dr. Kathryn Hamilton) also traveled to ICARDA in Aleppo, Syria during October 21-23 to attend a contract negotiation and drafting seminar conducted by Dr Sean Butler, CAS Honorary Fellow and law faculty at St. Edmund's College, the University of Cambridge. The purpose of the workshop was to help managers to understand the basic principles of different types of contract (*e.g.*, confidentiality, collaboration, research agreements). The workshop involved breaking agreements down into their core elements, to understand why all the elements are there, how they could be different, how they fit with the aims and the principles of AVRDC. The goal was that managers will understand contracts from a fundamental viewpoint, so that when they get a contract (or have to prepare one) they can do so more easily.

AVRDC will continue to refine the commercialization agreement. The whole process has taken much longer than anticipated partly hindered by the number of people involved and the internal approval steps needed along the way. Project team members need to reach an agreement before passing it on to their respective legal teams who only want to see the document in its final stages. This has proved to be very inefficient if the project member is not closely aligned with their legal support system. Currently the document has been modified by CAS on behalf of the public partners and we are waiting feedback from the private partner lawyers. Timing is becoming more of an issue as some project members are moving to other positions. We are still hoping to complete the agreement during the first quarter, 2008. It has been and continues to be, a huge learning process for AVRDC as the public and private goals of each entity attempt to align for their mutual benefit

<u>Objective 2</u>: Socio-economic studies on crucifer production and pesticide use and on the attitude of farmers and consumers on GM vegetables

<u>Activities proposed in the proposal</u>

Socioeconomic studies on production and protection of crucifers will be conducted on sites in India with high vegetable production based on a quantitative survey among 300 farmers including aspects such as attitudes on GM vegetables and pesticide use and costs. In addition, attitude of consumers on GM vegetables and pesticide in major Indian cities based on interviews with at least 600 households will be assessed. The baseline data generated during this project will also be useful in the future to study the impact of introduced dual gene Bt-transgenic technology for crucifer pest control. The results will be published in peer-reviewed journal.

<u>Milestones achieved in reporting period</u>

Output 2.a Socioeconomic studies on production and protection of crucifers based on a quantitative survey among 300 farmers and including aspects such as attitudes on GM vegetables and pesticide use and costs.

Milestones achieved

Data was collected between November 2006 and February 2007, based on a structured questionnaire that was translated into three local languages (Gujarati, Kannada and Bengali). As planned, 300 farmers were interviewed in three different states of India, namely Gujarat (100), Karnataka (100), West Bengal (100), and including a sample of 150 farmers each for cabbage and cauliflower production. Data imputation and cleaning were conducted between March and July 2007. Data was analyzed subsequently. One presentation based on the results was given during a CIMBAA meeting in London, from 21-22 June 2007 at the University of Greenwich, London, and one Working Paper has been prepared for publication. It is planned to prepare one publication for submission to a peer-reviewed journal during the next 6 months.

Summary of results

This study has attempted to analyze pesticide use in crucifer production in the three states of India. As could be expected from the sampling design of the study, crucifers play important role in farm livelihoods. Compared to other vegetables grown on the farm, the respondents faced more pest problems in cabbage and cauliflower, since insect resistant varieties are not available. Pesticide use is thus very common. Only ten farmers (3.3%) out of the entire sample of 300 farmers did not use any pesticide in their crucifer field. The share of farmers using pesticides was similar in all the three states. Farmers relied on pesticide stores for their information on dosage and correct pesticides to use. However, farmers were skeptical and approximately 23% of farmers applied a higher amount than the recommended dosage. The use of insecticides was more common than the use of fungicides. Approximately 50% of all farmers used fungicides, while 98% of farmers used insecticides. The mean number of pesticide applications per cropping cycle varied from 5.6 for cabbage in Gujarat, and 57.6 for cauliflower in West Bengal, with a maximum number of 248 applications in West Bengal. A total of 47 different active ingredients were being used in crucifer production. The use of hazardous pesticides [classified as Ia (extremely hazardous) or Ib (highly hazardous) according to WHO] was common and especially prevalent in cabbage production, where four out of the ten most commonly used pesticides belonged to these two groups. Of the entire sample, four out of 10 farmers sprayed at least one pesticide falling into this category. The share of pesticide cost in total cost was 14% for the entire sample, but with wide variations. It must be assumed that because of the relatively small cost factor involved (with the exception of cauliflower use in West Bengal), it will be difficult to convince farmers to use alternative pest control measures.

Nearly 65% of respondents did not use any protective measures while spraying, and this response rate was similar in the three states. Spillage of pesticide is a common occurrence. More than two-third of the sample (68%) have spilt pesticides on the body at one point in time. The reported practices regarding disposal of the empty containers were unsafe. The empty containers used by most of the respondents were thrown near the field. About two-third of the total sample reported to have had some health related symptoms due to pesticide application in the past five years. The farmers in West Bengal reported more symptoms than those in Gujarat and Karnataka. At the time of survey, 15% of the total respondents had symptoms due to pesticides use. Out of the total sample, 7% reported that they had serious pesticide hazard and all of them were hospitalized because of it.

There is a marked difference in the willingness to use alternative pest control measures among Karnataka and the other two states. In Karnataka, 75% of farmers registered their willingness to apply alternatives to pesticides. In Gujarat and West Bengal, the majority of the growers (87 and 96%, respectively) were not willing to use an alternative to pesticides. The overall attitude towards GM vegetables is positive. As expected, the awareness of GM crops among the farmers was very low. At the time of the survey, no GM vegetables were being cultivated in India. Only 7 farmers out of 300 knew about GM vegetables and 21% of respondents knew about other GM crops (especially Bt cotton). Prior knowledge was lowest in West Bengal. However, it was there that farmers showed the most positive attitude towards GM vegetables. The major advantage of GM vegetables is seen in that they could reduce production cost and increase productivity.

In conclusion, pesticide use is high but farmers have few alternatives. There is a need for development of resistant/ tolerant varieties. Since the share of pesticide cost in total production cost was small, it would be difficult to convince farmers to use alternative pest control measures. As attitude towards GM crops is highly location specific, a communication strategy regarding GM vegetables is important but may need to vary according to local conditions.

Output 2b. Attitude of consumers on GM vegetables and pesticide in major Indian cities based on surveys with at least 600 households assessed

Milestones achieved

As reported in the prior progress reports, survey activities were completed with 600 households from five urban locations in India, *viz*., New Delhi, Bengaluru (formerly Bangalore), Kolkata (formerly Calcutta), Kolar, and Barddhaman (Burdwan). One paper entitled *"Consumer Attitudes Towards GM Food and Pesticide Residues in India"* was submitted to the Review of Agricultural Economics and one presentation based on this paper has been presented as a contributed paper at the 9th Conference of the International Society for Ecological Economics, 15-18 December 2006, in New Delhi, India.

Summary of results

Bt vegetables have the potential to reduce pesticide use considerably. The GM technology entails not only clear agronomic advantages, but also benefits to consumers. Productivity increases will lead to a reduction in market prices for vegetables. In addition, lower pesticide residues could bring about advantages for human health. In this project, consumer attitudes towards the introduction of Bt vegetables have been analyzed, paying particular attention to the question whether awareness of and concerns about current pesticide residue problems influence the technology acceptance. The urban households in India are generally well aware of the health risks associated with pesticide residues in foods. The mean of WTP for residue-free vegetables is more than 50% above current market prices. This suggests that a reduction in pesticide residues through Bt technology could lead to substantial welfare gains for consumers. On the other hand, there are perceived risks and ethical concerns associated with GM foods, which cannot be ignored when analyzing public attitudes. Among the households surveyed, 68% showed a positive attitude towards the introduction of Bt vegetables, while only 17% were mildly or strongly opposing the technology. Better education and greater exposure to mass media seem to reduce acceptance in the Indian context, partly reflecting biased information flows. Furthermore, consumer's trust in the public food safety authorities matters: those who consider the authorities ineffective are less willing to accept GM foods. Nevertheless, the overall attitude towards GM foods in India is quite positive. The WTP analysis demonstrates that 55% of all consumers would purchase Bt vegetables even if they were sold at current price levels for conventional vegetables. At a price discount of 10%, more than 80% of households would purchase Bt vegetables. This high acceptance level bodes well for the future of this particular technology and other GM crop innovations to come. These results confirm those of previous studies showing that GM food acceptance is often higher in developing than in developed countries. One plausible explanation is that people in developing countries are generally poorer and sometimes food-insecure, so that they are more dependent on productivity-increasing agricultural technologies than better-off consumers in developed countries. However, consumer acceptance and WTP studies can always only provide a snapshot, which is based on consumers' information levels at a particular point of time. In most developing countries, including India, no GM food crops have been released so far, and related knowledge is still very limited. Once such crops are introduced, information levels will increase, but not in all cases will the additional information be objective. Depending on the success of different interest groups in spreading their subjective viewpoints, consumer attitudes could certainly change in either direction. The public media can and should play an important role in spreading unbiased information, a role which was not always fulfilled in the past.

Another interesting finding is that WTP for pesticide residue-free and WTP for Bt vegetables in India are negatively correlated, that is, people who are most concerned about pesticide residues are least willing to accept Bt food. This can be explained by general risk attitudes: for risk-averse consumers, a reduction in one risk cannot easily compensate for the perceived increase in another. More generally, consumers who are concerned about the potential risks of a new technology may undervalue tangible benefits. This could also have implications for second-generation GM crops, which primarily focus on consumer traits such as higher nutrient contents in foods or other health-improving ingredients. While it is generally believed that such second-generation traits might improve GM acceptance among those who currently oppose the technology, our results suggest that this might not always be the case. More research in this direction is certainly needed before conclusive statements can be made. A better understanding of how consumers value different types of risks and benefits can help improve the innovation process.

***Objective 3*: Testing of the dual gene Bt crucifers against secondary pests and parasitoids**

Activities proposed in the proposal

Crocidolomia binotalis, Hellula undalis, Spodoptera litura, Pieris rapae and Phyllotreta striolata are pests of secondary importance in most crucifer crops. Although DBM is the predominant pest in crucifers, it can be brought under reasonable control in highland or cool season production by a guild of mostly introduced parasitoids including, Cotesia plutellae, *Diadegma semiclausum, Microplitis plutellae, Oomyzus sokolowskii and Diadromus collaris present in the region. However, the secondary pests of crucifers often lack parasitoids or other soft control measures, thus vegetable growers rely on chemical insecticides to control these secondary pests which inadvertently kill the natural enemies of DBM.This results in resurgence in the DBM population. Hence devising effective pest management strategies for pests of secondary importance, which will reduce pesticide use, will also help to reduce the DBM population. Reduced DBM population will also lead to further minimize the possibility of resistance development in DBM and will prolong the usefulness of Bt-transgenic cabbage in pest control. We are hoping that one or both of the Bt genes intended for use in dual gene Bt-cabbage is found to be effective against the above mentioned secondary pests because of the wide host range of B. thuringiensis in Lepidoptera. Therefore, efficacy of various Bt-crystal proteins alone and in combinations will be investigated against the secondary pests.*

Milestones achieved in reporting period

As the elite event selection has not yet been completed, the Bt toxins that have been expressed in the dual-gene Bt brassicas were used for the toxicity assays in the laboratory. In addition, simulated field studies were carried out with available Bt formulations, especially Xentari. Xentari is a commercial Bt formulation based on the *B*. *thuringiensis* subsp. *aizawai* containing Cry1A and Cry1C. As there is no Bt formulation containing Cry1B and Cry1C, we had chosen Xentari for the simulated field studies. All the experiments were carried out at AVRDC – The World Vegetable Center in Taiwan, as well as at University of Agricultural Sciences (Bangalaore), Anand Agricultural University (Gujarat) and Visva Bharati (West Bengal) in India.

Susceptibility of the major insect pests on vegetable brassicas to Cry1B and Cry1C

Two Bt toxins *viz*., Cry1B and Cry1C had been tested for their toxicity against diamondback moth (DBM), *Plutella xylostella*; cabbage head caterpillar (CHC), *Crocidolomia binotalis*; cabbage web worm (CWW), *Hellula undalis*; imported cabbage worm (ICW), *Pieris rapae* and common army worm (CAW), *Spodoptera litura* at AVRDC, Taiwan. DBM was susceptible to both the toxins, Cry1B and Cry1C. The other two major insects, CHC and CWW were also susceptible to both the toxins. However, the ICW was susceptible only to Cry1B and it was highly insensitive to Cry1C. Finally, the CAW was not sensitive to both the toxins.

DBM is the major insect pest on the vegetable brassicas in India. The bioassays revealed that the DBM in West Bengal was highly susceptible to both Cry1B and Cry1C toxins. Gujarat population was also equally susceptible as West Bengal population, and the Karnataka population required slightly higher toxin doses to be susceptible. Comparatively, the DBM in India was more susceptible to Cry1B than the Taiwan population. However, both populations were equally susceptible to Cry1C.

CHC, CWW, ICW and CAW are highly sporadic on vegetable brassicas in India, and hence the bioassays were not done for these insects. However, the susceptibility of CHC in Karnataka was evaluated. The general baseline susceptibility data revealed that this insect was susceptible to both Cry1B and Cry1C, and its susceptibility to both toxins was comparatively higher than the Taiwan population. In addition, the susceptibility of CAW was assessed in Karnataka as well as in Gujarat. Just like the Taiwan population, CAW was least sensitive to both toxins in India. Hence, care should be taken while introducing the dual-gene Bt brassicas in India and proper integrated pest management package should be in place to manage CAW which may not be controlled by dual-gene Bt brassicas.

Impact of Bt on the population dynamics of lepidopterans, sucking insects, natural enemies and soil arthropods in vegetable brassica fields

1. **Impact on target (lepidopterans) and non-target insect pests (sucking and leaf-feeding insects)**

(i) Taiwan

To understand the population dynamics of various insect pests in dual-gene Bt brassicas, a simulated field study was organized. As we did not have any Bt commercial formulations containing the Cry1B toxins, we used the commercial formulation, *Xentari*, which is based on *B. thuringiensis* subsp. *aizawai* and contains Cry1C as one of the toxins. This formulation was compared with the standard insecticide, cypermethrin (a synthetic pyrethroid), which was being used by vegetable growers. Two field trials were conducted, with the first during Spring 2006, and second during Autumn 2006. The results indicated that DBM populations were generally lower in the Bt treated plots, although there was no significant population differences between the control and Bt treated plots. However, the pyrethroid pesticide could not control the DBM, which might be due to the resistance in DBM to pyrethroid insecticides. Bt was also effective in controlling imported cabbage worm (ICW), *Pieris rapae*; cabbage head caterpillar (CHC), *Crocidolomia binotalis* and tomato fruit worm (TFW), *Helicoverpa armigera*. But it did not significantly control the beet armyworm (BAW), *Spodoptera exigua*; common armyworm (CAW), *S. litura* and cabbage looper, *Trichoplusia ni*. It did not work against the sucking insects, especially striped flea beetle as anticipated. Hence, the dual-gene Bt brassicas may not control the sucking insects, and few other lepidopterans such as BAW, CAW and *T. ni* which might need the IPM package to provide a complete control and thus reducing the pesticide abuse.

(ii) India

(a) Karnataka

The field experiment was carried out on cabbage at Manchinabele village in Kolar district of Karnataka during July – November, 2006. Bt was compared with Spinosad (commonly used pesticide) and an untreated check. The mean population of DBM was continually lesser than one larva per plant throughout the cropping season in Bt treated plots whereas untreated plots had the highest number of DBM population followed by pesticide-treated plots. The mean population of the CAW was less than 0.1 larva per plant in Bt treated plots throughout the cropping season whereas the population was quite higher in pesticide-treated plots (0.6 larva) and untreated plots (0.9 larva). Although the CAW was not susceptible to Cry1C, *Xentari* may control it because it contains Cry1A in addition to Cry1C. The CHC was highly sporadic and it was under check in Bt- and pesticide-treated plots. The incidences of aphids and whiteflies were higher after 19 days of transplanting (DAT) of cabbage and they reached the peak at 61 DAT in the pesticide treated plots. The incidences of these sucking insects were lower in Bt- and untreated plots. In Bt- and untreated plots, the natural enemy population which could keep the aphid and whitefly population under check may be quite higher, whereas in the pesticide-treated plots the pesticide might have killed these natural enemies and thus increased the incidences of the aphid and whitefly. Bt treated plots produced the highest mean head weight (2.73 kg), which was statistically significant than the pesticide-treated plots (2.52 kg) and untreated check plots (1.22 kg).

(b) Gujarat

The first field trial was conducted on cabbage in Navli village of Anand district during October 2006 – January 2007. Bt and pesticide (cypermethrin) treated plots recorded lower DBM population than control. Bt treated plots had 0.17 larvae per plant, which was significantly lower than cypermethrin (1.19 larvae per plant). There were significant differences in the larval population of CAW among the treatments. The pesticide (cypermethrin) plots recorded the lowest larval population (0.40 larvae per plant) and were on par with Bt plots, which recorded 0.60 larvae par plant. Thus, Bt is also effective against CAW in Gujarat. Bt plots recorded a significantly lower population (0.17 larvae per plant) of tomato fruit worm (*Helicoverpa armigera*) than control plots (0.48 larvae per plant) after two rounds of spraying, although it was on par with the pesticide plots which recorded 0.33 larvae per plant. However, Bt plots recorded significantly the lowest larval population and showed its superiority against TFW after third spraying. Bt plots recorded 30.43% higher marketable heads of cabbage over control as compared to 21.74% in cypermethrin plots over control.

The second field trial was conducted on cauliflower in Navli village of Anand district during December 2006 – March 2007. Bt plots were found to be superior and significantly recorded the lowest (1.28 larvae per plant) DBM population than cypermethrin plots (4.75 larvae per plant) and control (7.78 larvae per plant). The efficacy of cypermethrin plot was on par with control. Statistically significant higher marketable cauliflower heads were recorded in Bt plots in terms of number (23.02) and weight (13.86 kg) than in cypermethrin plots (17.99 and 10.32 kg). The lowest marketable heads were harvested in the untreated control plots (13.49 and 7.62 kg). Similar results were obtained in the third field trial, which was conducted on cabbage in AAU campus, Anand during December 2006 – March 2007. Bt plots were found to be superior and recorded the significantly lowest (0.60 larvae per plant) DBM population than cypermethrin plots (0.92 larvae per plant) and control (1.72 larvae per plant). Significantly higher (24.81 t/ha) yield was recorded in Bt treated plots than cypermethrin (22.18 t/ha) treated plots.

(c) West Bengal

Two field trials were conducted. The first field trial was conducted on cabbage in Bahadurpur village of Bolpur block during October 2006 – January 2007. The second field trial was also conducted on cabbage in Junnatpur village of Suri block 1 during February – April 2007. Both Bt and the pesticide (chlorpyriphos) treated plots were significantly superior over untreated control in reducing the larval population of DBM at each date of observation after spraying. The efficacy of Bt was on par with the pesticide. In comparison with the control, Bt provided 69.84% reduction on 7 days after spraying. In contrast to Taiwan and Bangalore, Bt had effectively controlled the CAW in West Bengal, which had indicated that the CAW population in West Bengal was highly susceptible to Bt. This was also confirmed in the laboratory bioassays where the LC_{50} values for the 2nd and 3rd instar larvae of CAW were 11503.72, 16645.63 IU/ml respectively. The population of CHC was below the economic threshold level in all the plots. There was no significant difference in the population of flea beetle (*Monolepta* sp.) among the treatments. Surprisingly, the cabbage aphid (*Brevicoryne brassicae*) population was significantly higher in the untreated control, and the Bt as well as pesticide treated plots had no aphid infestation. Although chlorpyriphos, an organophosphorous pesticide, could control the aphids, the causes for the absence of aphids in Bt treated plots were unclear as the Bt could not control the aphid. Maximum number of grade-1 heads was obtained from Bt treated plots (64%), followed by pesticide plots (55%) as compared to only 33% in untreated control. Total income of Rs 103,958 (US$ 2650), Rs. 98,210 (US$ 2500) and 82,419 (US$ 2100) per hectare were recorded in Bt, pesticide and untreated control plots, respectively.

2. Impact on natural enemies

(i) Taiwan

The larval parasitoid of DBM, *Cotesia plutellae* was commonly found in the low land cabbage production systems in Taiwan. There was a significant difference in the level of parasitism by *C. plutellae* in Bt treated plots (76%) followed by untreated control plots (63%) and pesticide treated plots (55%) in the early season. However, there was no significant difference in the level of parasitism among the treatments during mid and late seasons. This may be true because of the enhanced tolerance of *C. plutellae* to the commonly used pesticides in this region. In conclusion, Bt did not have any adverse impact on the parasitoid.

(ii) India

(a) Karnataka

During 23, 46 and 71 days after transplanting (DAT), there was no drastic difference in the predation of eggs/larvae and in the rate of parasitization of DBM larvae between the Bt treated-, pesticide treated-, and control plots.

(b) Gujarat

The Bt treatments did not show any adverse effects on the predator, syrphid fly in the field. Bt plots recorded a higher larval population (0.35 larvae per plant) than cypermethrin (0.17 larvae per plant) and it was statistically significant. Hence, Bt was comparatively found safer than cypermethrin to syrphid fly in cauliflower. A higher number of larval parasitoid *C. plutellae* emerged out from the DBM larvae collected in the control plot (28.77%), which was on par with the Bt plots (27.40%). Cypermethrin was found to be more toxic to *C. plutellae* and recorded significantly the lowest parasitism (15.27%). Overall, Bt was found to be safer to *C. plutellae* than cypermethrin.

Similar results were obtained in another field trial. Significantly higher larval population of syrphid fly was observed in the control (0.21 larvae per plant) plots, which was on par with the Bt treated plots that recorded 0.14 larvae per plant. Significantly lower population was recorded in cypermethrin plots (0.08 larvae per plant). Significantly higher number of *C. plutellae* emerged out from DBM larvae collected in the Bt treated (19.18%) and control (22.60%) plots, than in cypermethrin treated plots (10.30 %).

(c) West Bengal

Population of predatory coccinellid beetles (*Menochilus, Micraspis, Schymnus and Harmonia* species) was found to be reduced by 23.43% in Bt treated plots after 14 days of spraying, and it was reduced by 6.37% in untreated control. Chlorpyriphos emerged as highly toxic to coccinellid predators because its population was reduced by 32.41%. The reduction in the predator population in Bt treated plots might be due to the reduced host (lepidopterans) availability. However, this was not the case in spider (*Lycosa psedudoannulata*; *Oxyopes* sp and *Argiope* sp) populations. Bt treated plots recorded 14.23% reduction and it was on par with the untreated control which recorded 11.89%

reduction. But, chlorpyriphos had significantly reduced the spider population by 29.75%. Therefore it can be concluded that Bt is comparatively safer to non-target beneficial fauna than the conventional pesticides.

3. Impact on soil arthropods

i) Taiwan

As plastic mulch had been used to control the weeds, the impact on soil arthropods were not monitored in the field trials at AVRDC.

ii) India

(a) Karnataka

On 23 DAT, the number of arthropod groups recorded was 10, 8 and 9 in Bt-, pesticide- and control plots, respectively. The control treatment recorded a higher species evenness as compared to Bt- and pesticide plots, while Bt plots recorded a higher species diversity and richness. During 46 DAT, the numbers of arthropod group recorded were 10, 10 and 11 in Bt-, pesticide- and untreated control plots. The relative abundance in terms of species evenness and species diversity was higher in Bt treated plots, while untreated plots measured higher species diversity than pesticide treated plots. On 71 DAT, Bt-, pesticide- and untreated plots recorded 8, 9 and 11 arthropod groups, respectively. Bt treated plots recorded a higher species evenness followed by untreated control and pesticide treated plots, whereas untreated plots recorded a higher diversity and richness of species.

(b) Gujarat

The arthropods population entrapped in Bt was higher than that entrapped in pesticide and control. The diversity of arthropods included coleopteran, dermaptera, hymenoptera, lepidoptera and arachnida. Significantly more arthropods (9.81 arthropods/trap) were entrapped in Bt treated plots followed by pesticide (6.95) treated plots and control (4.57).

(c) West Bengal

Abundance of spider (*Lycosa and Clubiona spp*), ground beetle (*Ophionea sp*) and earwig (*Dermapteran sp*) were significantly higher in untreated control plots, followed by plots treated with Bt. The pesticide treated plots had a lower population of these soil arthropods. However, the population of field cricket (*Gryllid sp*) was higher in untreated control plots, followed by pesticide treated plots. Bt treated plots recorded the lowest population, which needs to be validated. In general, the arthropod populations were higher during the early and mid-seasons than the late season.

***Objective 4*: Meeting of all participants after twelve month to discuss the results of the socio-economic studies and the progress of the legal framework development**

Milestones achieved in reporting period

The mid-term progress and planning workshop was not organized after 12 months of the project as it was originally proposed to discuss the results of the socio-economic studies and progress of the legal framework development. However, it was held on 21-22 June 2007 at University of Greenwich, London. In addition to the CIMBAA International Advisory Panel members, the CIMBAA Steering Committee and Project Team members have participated in this meeting. Prof. Matin Qaim (University of Hohenheim) presented his findings on the consumer attitudes towards GM vegetables in India. Dr. Srinivasan (AVRDC – The World Vegetable Center) presented the current pesticide use patterns and farmers' attitudes towards GM vegetable brassicas in India. The development of CIMBAA commercialization agreement was discussed in detail on the afternoon of 22 June 2007. Dr. Victoria Henson-Apollonio from CAS led the discussion. Most issues indicated by the partners on this draft commercialization agreement were discussed in detail, and consensus had been drawn on most issues. The CAS lawyer for AVRDC has been revising this draft commercialization agreement

Future work plan

There are about 130 species and 25 genera of Brassicaceae in India. Most of the species are weeds which could be found in the vicinity of cultivated brassicas. They serve as wild host plants to the insects which would commonly occur in cultivated vegetable brassicas. Wild host plants are especially important for the sustenance of DBM early in the year in temperate climates before cultivated *Brassica* plants are planted. For instance, *Thlaspi arvense*, *Barbarea vulgaris* and *Raphanus raphanistrum* were recorded as host plants of DBM. These insect pests assume the role of 'weed-killers' on these wild brassicas and they keep the weedy brassicas under control. These weedy brassicas may become 'super-weeds' due to out-crossing with transgenic vegetable brassicas if they could produce hybrids under natural conditions. This is especially important only in seed producing regions, where the vegetable brassicas are being allowed to flower. Hence, it is proposed to develop a list of commonly occurring weedy brassicas in the major vegetable brassica seed production regions of India, and their host status to the selected lepidopterans on vegetable brassicas. This would provide an answer to the questions regarding the possible creation of 'super-weeds' due to the introduction of dual-gene Bt brassicas in India. A proposal has already been submitted to Vater und Sohn Eiselen-Stiftung, Ulm, Germany with a budget request of € 54,525 to study these issues.