Chickpea (*Cicer arietinum*) is the most important pulse crop of India and is one of the major sources of proteins for the vegetarian population. One very serious pest of chickpeas is pod borer (*Helicoverpa armigera*). Non-availability of resistant sources hinders the development of resistance to this serious insect pest in chickpeas using conventional breeding. With the development of transformation systems it is now possible to incorporate insect resistance genes from alien sources into this important grain legume. Keeping this in mind, the project aimed at 1) the development of an efficient *in vitro* regeneration and transformation protocol for the Indian cultivars of chickpea and 2) development of transgenic chickpeas resistant to pod borer.

During the first programme phase, several transgenic lines were developed using chimeric Bt- Cry1Ac genes. PCR analyses confirmed the presence of the transgene in these lines as well as in their progeny. Protein dot blot and Western blot analyses confirmed the expression of the transgene in T1 progeny of four independent lines. However, insect bioassays using the T1 progeny of these lines did not show resistance against the target insect (pod borer). Thus it was essential to produce many more new Bt transgenic lines using one or more chimeric Bt gene(s) during the second programme phase. During the Phase 1 chimeric Cry1Ac genes were reconstructed using Cre-Lox excision system in order to facilitate elimination of marker gene from segregating progeny. However, after looking into the possible IPR (intellectual property right) issues related to the use of Cre-Lox system (owned by DUPONT, USA), emphasis was put on the reconstruction of the Bt genes using a twin binary vector system (available from Dr Higgins at CSIRO, Canberra), in order to eliminate marker gene from segregating progeny.

During the second phase, we have successfully sequenced the coding region of the two Bt genes. Clones with Cry1Ac coding sequence were obtained from Dr TJ Higgins of CSIRO, Canberra and Dr A Kumar of IARI, New Delhi and a clone with Cry2Aa coding sequence was obtained from Dr Kumar. We compared these sequences with patented versions, in order to determine if there was any difference. Differences were observed both in nucleotide sequences and amino acid sequences when Cry1Ac sequences of Prof Higgins’ clone and Dr Kumar’s clone were compared to Monsanto’s patented version. These differences were corrected using genetic engineering techniques, and the new corrected Cry1Ac coding sequence was 100% similar to that of patented version. In the case of Cry2Aa gene the blast search indicted that the clone obtained from Dr Kumar had the sequence, which matched 100% to that of patented version and thus did not need any correction. Considerable effort was devoted to this phase of the work in order to obtain Bt chickpeas with versions of the Bt proteins that have already been approved for release in several countries. We then reconstructed Bt genes (Cry1Ac and Cry2Aa) in a twin binary vector.
We transformed chickpea using a recombinant binary vector (pBK202) harbouring the Cry2Aa gene in one T-DNA and nptII as selection marker gene in the other T-DNA. Fifteen nptII positive lines were established in soil in the greenhouse. Of these, thirteen expressed Cry2Aa toxin in the leaves of T₀ plants and eleven of these transmitted the gene to the next generation. Molecular analyses of segregating progeny suggested that segregation of selection marker gene (nptII) from Cry2Aa gene was possible but with a very low frequency. Five lines transmitted the cry2Aa gene consistent with an expected 3:1 segregation. Western analysis of proteins from different organs showed accumulation of Cry2Aa protein in all the green parts as well as in petals and cotyledons. Insect bioassays using the progeny of selected lines showed differential resistance to pod borer (Helicoverpa armigera) larvae, depending upon the level of expression of the Cry2Aa protein. A high expressing line (BS6H) was found to confer near complete protection (over 98 % larval mortality) against the target pest pod borer. We are attempting to obtain homozygous plants in this line. We were unable to analyse enough T₁ plants as many T₁ seeds of this line did not germinate. All T₁ plants still segregated at the T₂ generation. Western blot analyses of seed protein in this line confirmed presence of Cry2Aa toxin in mature seeds. Any possible negative correlation between Cry2Aa toxin content of seeds and germination capacity of seeds needs to be further investigated.

The binary recombinant vector with both Cry1Ac and Cry2Aa genes in a tandem orientation in one T-DNA (pBK201) was used for transformation of several chickpea cultivars or lines (Lasseter, Jimbour, Howzat, BG1101 and BG286). A total of 41 co-transformed plants were found to be positive by PCR for both the genes. Molecular analyses (Western blot) confirmed low level of expression of the transgenes in all PCR positive lines. An RT-PCR experiment also confirmed low level of mRNA for both genes. Thus these lines were not subjected to in vitro insect bioassays assuming that they will not confer resistance against pod borers. There is a need to reconstruct this vector for future use in chickpea transformation.

Emphasis is now given on transforming chickpeas with a Cry1Ac gene alone. So far we have not been able to generate a stable line using this construct. In the longer term we propose to cross the best Cry1Ac line with the best Cry2Aa line. A comparative study will eventually be made between two Bt lines and single Bt lines in regard to their resistance against insect pests.