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Full Length Research Paper

Transformation of chickpea lines with *Cry1X* using *in planta* transformation and characterization of putative transformants T1 lines for molecular and biochemical characters

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The transgenic plants of the chickpea (*cicer arietinum*) cv. KAK-2 expressing a Bt gene, were generated using an *Agrobacterium tumefaciens* mediated transformation system. A tissue culture-independent transformation method, *in planta* which targets the *A. tumefaciens* to the apical meristem was used in this study. The protocol involves *in planta* inoculation of the embryo axes of the germinating seeds and allowing them to grow into seedlings *ex vitro*. Polymerase chain reaction (PCR) analysis indicated the putative transgenic nature of theT1 generation plants. Bioassays against major pests of the chickpea, *Helicoverpa armigera* revealed several T1 plants that perform well against the larvae. This revealed that 43 of T1 plants harbor the transgene. The seeds of 43 T1 plants were allowed to continue into the next generation amplified gene of interest in most of the plants tested. Enzyme Linked-Immuno Sorbent Assay (ELISA), PCR and Bioassays was used to identify the high expressing plants. These results suggest that the Bt gene was functional in the transgenic chickpea and was being expressed. The study also showed that the chickpea plants harboring the cry1X gene were resistant to *Helicoverpa armigera.*

Key words: Chickpea, *Helicoverpa armigera*, transformation, in-planta, tissue culture, synthetic cry gene.

INTRODUCTION

Chickpea, *Cicer arietinum* (L.) (Fabaceae) is one of the 3rd most important grain legumes in the world. Chickpea seeds contain high quality, easily digestible protein (25%) and carbohydrates (20%) making it an important source of protein for the vegetarians of the indin and thus it is also called “poor man"s meat (Bahl and Salimath, 1996)”. The demand for chickpea, worldwide, stands around 8.2 million tonnes per year and is expected to increase to around 11.1 Mt per year by 2010 (Singh, 1987). Among several pests of the crop, the pod borer, *Helicoverpa armigera*, is the most important and is estimated to cause up to 40% yield loss (Rahman, 1990). One of the practical means of increasing crop production is to minimize the pest-associated losses. The input costs for the management of these pests were estimated during 2000 at $ 32 billion annually so; alternatives to chemical control of insect pests have become an urgent necessity. Among the various options available is a novel delivery system of insecticidal toxin through crop plants that is, transgenic crops with suitable insecticidal genes. A classic and highly successful example is the *Bt* cotton plants in India which leads extensive use of *Bt genes* to develop insect resistant transgenic crops (Perlak et al., 1990). The single gene controlled δ-end toxins of *Bacillus thuringiensis,* widely labeled as *cry* genes or *Bt* toxins, were shown to be amenable for isolation and development of transgenic insect resistant crops (Hilder et al., 1987).

Thus a new avenue in the pest management of particularly Lepidopteran pests has been opened. The method is an elegant and perhaps the most effective delivery system for *Bt* toxins, that apparently provides a relatively long lasting and seed borne solution for the management of Lepidopteran pest (Tabashnik et al., 2003). The natural mechanism of *Agrobacterium*

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mediated gene transfer was the first successful plant transformation system, making the breakthrough in plant genetic engineering in 1987 (Hilder et al., 1987). Therefore, the synthetic constructs that incorporate the suitable elements of a number of Bt genes may provide protection against a number of crop pests besides being more effective against known susceptible pests (Honee et al*.,* 1990). Cry1X is a synthetic construct designed and developed by Indian Agricultural Research Institute (IARI), New Delhi and incorporates the elements of cry1Ac, cry1Ab, cry1Aa3 and cry1F. The novel construct, having been designed to act on a wide variety of Bt receptors, is likely to be effective against a range of lepidopteran caterpillars including some of the most difficult to manage such as, *H. armigera* and *Spodoptera litura.* Many recalcitrant crops such as chickpea that are not easily cultured under *in-vitro* conditions can now be effectively transformed using the *Agrobacterium tume-faciens* mediated *in-planta* transformation technique (Rohini and Rao, 2000a, b, 2001).

MATERIALS AND METHODS

Plant material and preparation of seedlings for transformation

Chickpea (*C. arietinum* L.) variety KAK-2, is one of the leading varieties in Karnataka and was used for transformation studies. This variety has extra large pod size with to fusarium wilt resistance. Seeds of chickpea (cv. KAK-2) were soaked in water over night and later surface sterilized with 0.1% mercuric chloride for 5 to 7 min, followed by thoroughly rinsing with sterile water. The seeds were later placed for germination in pertriplates at 30°C. The seeds were procured from the Zonal Agricultural Research Station, GKVK, University of Agricultural Sciences, Bangalore.

Bacterial strains and vectors

The A*grobacterium* strain EHA-105, harbouring the binary vector pBinAR with the gene of interest*cry1X*, was procured from Dr. P. Ananda Kumar, Principal Scientist, IARI, New Delhi. In this construct *Neomycin phosphotransferase* (*nptII*) gene was used as the marker gene while 35s promoter of CaMv as promoter and *nos* as terminator sequences. This construct in EHA-105 was used to transform chickpea (Figure 1).

Preparation of *Agrobacterium* culture for the transformation and recovery of transformants

A single colony of *Agrobacterium* harboring recombinant binary vector was grown in Lysogeny broth medium containing 50 µg/ml kanamycin overnight at 28°C on a shaker. The bacterial cells were later resuspended in Winan"s AB medium (pH 5.2) and grown for 18 h at 28°C on a shaker. Wounded tobacco leaf extract was kept overnight and after 18 h it was added to this suspension and incubated for 6 h on the shaker. Although many protocols can be employed for the transformation of plants, in the present study, *Agrobacterium* mediated *in planta* transformation protocol was employed due to the ease of the technique and the expertise available. Using these transformation protocols, kabuli chickpea variety KAK-2 was transformed with *cry1X* gene. The embryo axes were randomly pricked 4 to 5 times with a sterile sewing needle of 28 gauges and kept in the suspension of *Agrobacterium* in Winan"s AB medium. The infection was carried out by gentle agitation at 28 to 30°C, for 1 to 2 h and then these seedlings were blot-dried and washed thoroughly with 500 µg/ml of cefotaxime for 18 h and placed on autoclaved soilrite for further growth under aseptic conditions in capped bottles. After 5 to 6 days, the germlings were transferred to soil rite in pots and the seedlings were allowed to grow under growth room conditions for at least 10 days and later they were transferred to green house. The growth chamber was maintained at 26 to 28°C fewer than 14 h photo period with fluorescent light of intensity 35 μmol $m^{-2}s^{-1}$. The plants thus raised were T0 plants which were chimeric. The seeds from T0 plants were sown to get T1 plants.

Isolation and identification of the plasmid PBin-Bt8 in the *Agrobacterium*

The construct was ascertained primarily by culturing the labeled strain on kanamycin loaded at the rate of 50 µg/ml of agar or broth medium. Growth of the bacterium is an indirect indicator of the transgene in the bacterium. The plasmid deoxyribonucleic acid (DNA) was then isolated following the protocol (Sachdev et al., 2003). The isolated plasmid DNA was run on a 0.8% agarose gel to discern the presence of the transformed plasmids to confirm the existence of the *cry1X* gene construct in the *A. tumefaciens* culture of the strain EHA105.

Molecular analysis of transformants

Tissues from the progeny plants were analyzed for the presence of the introduced genes. Genomic DNA was isolated following the procedure of Dellaporta et al. (1983) from the fresh leaf tissue of the greenhouse-grown T1 generation plants that was used for PCR with individual plant samples and western blot analysis.

Enzyme linked immunosorbant assay

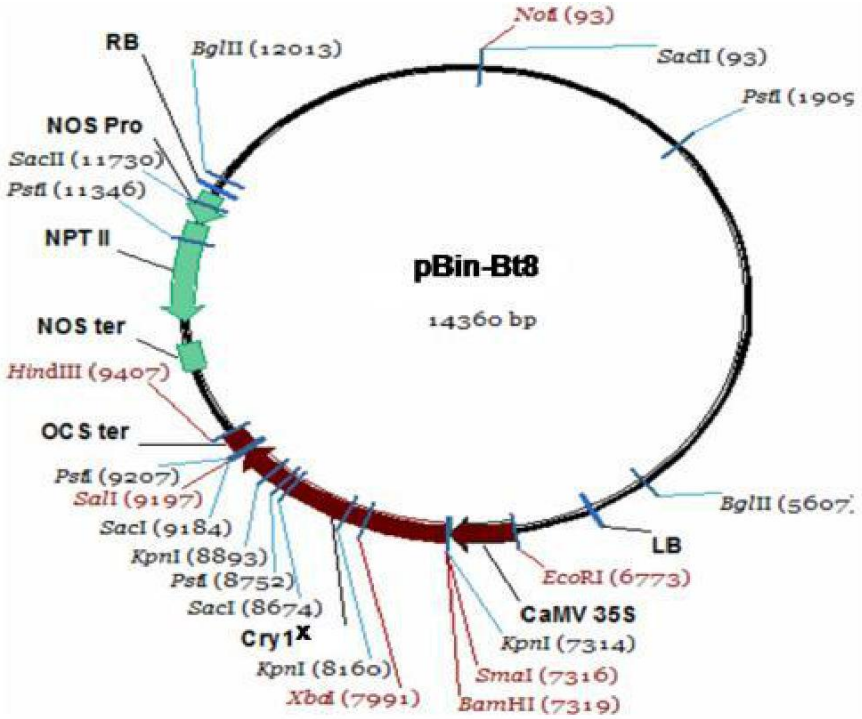
ELISA qualitative ELISA was used to check the cry protein produced in the transgenic chickpea plants. Cry1Ab/cry1Ac plate kit (Envirologix Inc, Portland, USA) was used for this purpose. The Sandwich ELISA was performed according to the manufacturer's instructions.

PCR analysis of T1 transformants

Seeds from each individual plant were maintained as separate lines. T1 chickpea plants were grown in green house following recommended package of instructions (Anonymous, 2000) and the plants were labeled. PCR was performed to confirm the presence of the gene in the plants that were selected to be advanced further. PCR was performed to amplify the 750 bp npt11 gene fragment in the putative transformants. In order to amplify the npt11 gene fragment, PCR was initiated by a hot start at 94°C for 4 min followed by 32 cycles of 1 min at 94°C, 1 min 30 s at 58°C and 1min 72C. PCR was also performed with the gene specific primers (cry1X gene) to amplify a 901 bp fragment (Figure 2). The condition for the reaction was the same as discussed earlier. The product was run on a 1% agarose gel.

Western blot analysis to confirm cry protein gene

To analyse total genomic DNA the protein samples were prepared and separated by sodium dodecyl sulphate-polyacrylamide gel



Binary Vector	:	pBinAR (11.0kb)
Insert	:	*cry1X* (1.9 kb)
A. tumefaciens	:	EHA 105
Bacterial selection	:	Kanamycin (50 µg/ml)

Figure 1. The genomic map of the plasmid pBin-Bt8 showing the insert and the restriction enzymes site.

electrophoresis (SDS-PAGE). Transfer stock was prepared by stacking six pieces of whatman no1 filter paper one over another followed by nitrocellulose membrane, gel and over the gel another six pieces of filter papers were placed. Electro bolting was done for 2 h at 50 volts and 45 mA current, and then keep the membrane at 4°C for overnight. Next day, membrane should be placed in a blocking solution for 1 h and wash with phosphate buffered saline tween-20 wash solution. Keep the membrane in a primary antibody solution for 1 h, after 1 h wash the membrane with wash solution and again keep it in the secondary antibody solution for another 1 h. Finally wash with Phosphate Buffered Saline Tween 20 (PBST) solution and soak the membrane in a substrate solution which develops color in the binding sites of primary and secondary antibodies after 30 min. Stop the reaction by washing with water and dried under dark condition.

In vitro insect bioassay

All bioassays were performed on detached, fully expanded, chickpea leaves. Two trifoliate leaves were collected from each selected plant and washed with distilled water. The leaves were wiped clean of all dirt and other debris. The stalks of the leaves were wrapped with wet cotton pieces and placed in a plastic container. Ten neonate larvae of *H. armigera* were released on to each leaf. Observations were recorded daily for a period of four days on the number of dead and live larvae, and percentage of leaf damage and the leaf condition. The containers were wiped clean daily.

Statistical analyses

Means and standard deviations were worked out for all the values to show the mortality and leaf damage. The mean values of all the plant parameters were subjected to analyse the variance (Sokal and Rohlf, 1969). Scatter plot and frequency distribution graphs were necessary for representing the data and were plotted using micro soft (MS) excel. The percentage leaf damage caused by test insect were worked out and the values were angular transformed, then subjected to single factor analysis of variance (ANOVA) to compare the means.

RESULTS

In planta transformation of chickpea variety KAK-2 with cry1X gene and analysis of the T1 generation plants

Approximately 60 seedlings were subjected to *in planta*

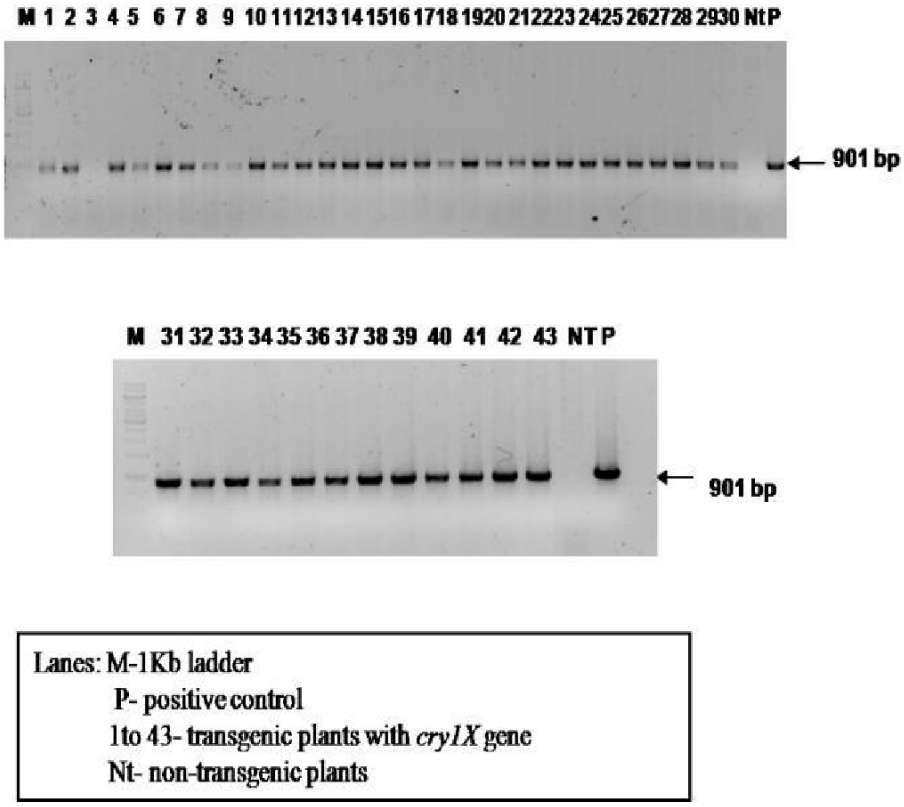


Figure 2. PCR amplification of selected transgenic plants over expressing cry1X gene of T_1 generation KAK-2 chickpea with cry1X gene specific 901 bp primers. Bands along the indicated arrow suggested positive sample.

transformation. 44 of these plants survived after shifting to the pots in the green house. After 7 to 8 days, the seedlings were transferred to soilrite in glass bottles and the seedlings were allowed to grow under room conditions for 10 days and later they were transferred to pots and kept in green house. Under green house condition, the plants showed reasonably good growth and development. Plants in this generation represented the T_0 generation, which contained the putative chimeric transformed plants. Seeds were harvested and used for raising the T1 generation plants. As many as 1200 seeds were harvested from 44 primary transformants out of which 1060 T1 plants were established in the green house among these 275 plants were selected for analysis.

Analysis for the presence of protein using ELISA

It was observed that the *cry1X* protein could be detected in 69 of the 275 plants by ELISA. Thus the results confirmed the expression of the transgene in some of the T_1 generation KAK-2 chickpea plants with *cry1X* gene. The *cry1X* protein detected by ELISA was calibrated and presented in microgram per gram tissue. Among these 69 plants, content of the toxin protein varied from 0.257 to 10.77 µg/g of plant tissue 4 plants were showing high protein content. The means for toxic protein in transgenic plants (3.15 ± 3.09) was found to be significantly different from those of the wild types (0.16 ± 0.20) as compared by t test ($t = 3.46$; $p < 0.01$) (Figure 3).

Molecular analysis by individual plant PCR

Plants that responded to ELISA were chosen for the identification of transgene among the putative transformants. Plant genomic DNA was extracted by cetyltrimethylammonium bromide (CTAB) method following the procedure of Dellaporta et al. (1983), a standard protocol that is commonly employed for DNA extraction. PCR analysis with *nptII* specific primers of 69 individual plant DNA samples revealed the presence of *N. phosphotransferase* gene (around 750 bp DNA fragment) in 43 individual plant DNA samples out of 69 verified (Figure 4).

Western blot analysis of transgenic chickpea harboring*cry1X* protein

Western blot analysis of transgenic chickpea using the

Figure 3. ELISA plate showing cross reaction of cry protein with antibody.

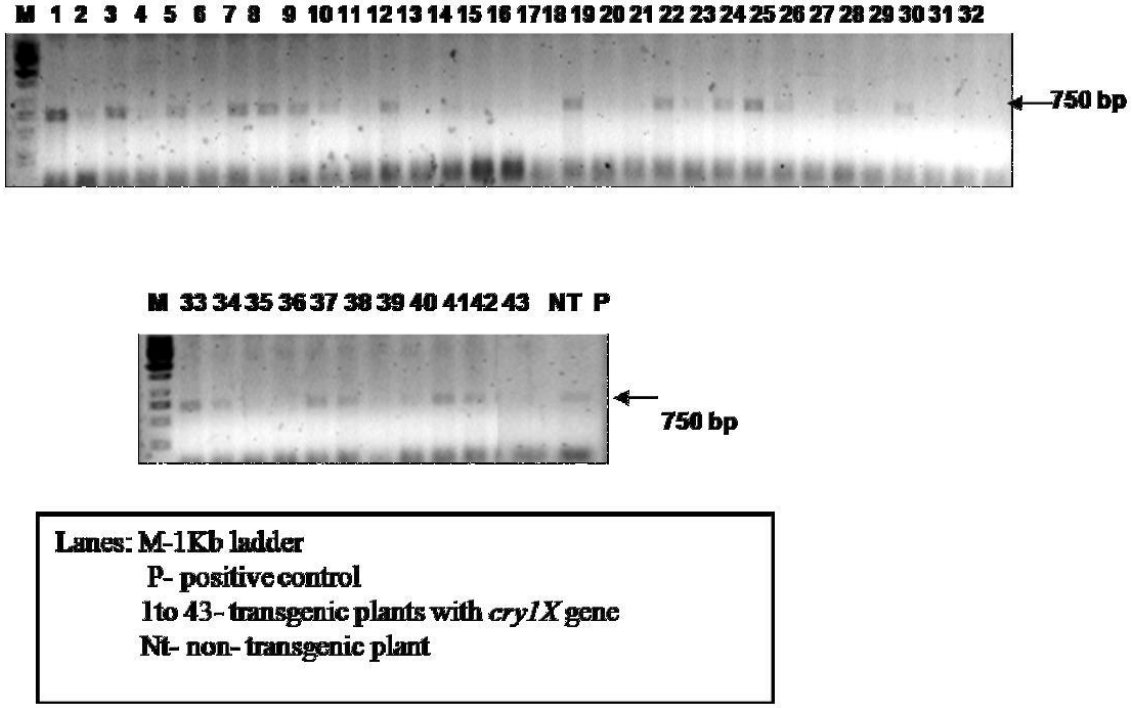
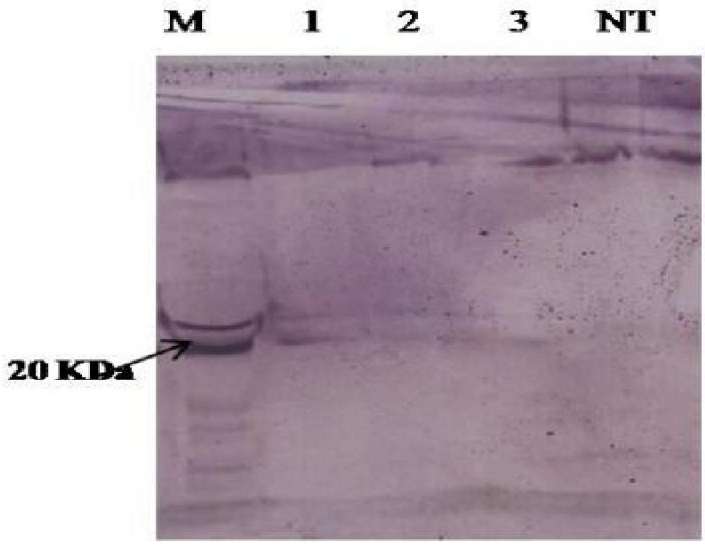


Figure 4. PCR amplification of selected transgenic plants over expressing cry1X gene of T_1 generation of KAK-2 chickpea with *nptII* 750 bp primers. Bands along the indicated arrow suggested positive sample.

antibody against the cry protein gave an indication that the plants were expressed as cry protein. Three plants were verified for the purpose and two of these clearly indicated the 20 kDa protein band depicting the



M- Marker 20KDa
1 to 3- transgenic plants
Nt – non- transgenic plants

Figure 5. Western blot anaysis of cry1X gene in transgenics (T_1) of KAK-2 chickpea.

expression of cry protein in transgenic plants (Figure 5).

Laboratory bioassay of *cry1X* transgenic plants

The efficacy of the cry gene product was tested against pest of chickpea *H. armigera.* Leaves from 4 to 6 week old chickpea plants, that were positive from PCR analysis, were taken for bioassay. Ten neonate larvae were loaded per leaf and were monitored for 96 h. Mortality and percent leaf damage were recorded to assess the effect of the protein on the larvae. Concurrent bioassays were also run on the wild type plants (Figure 6). The transgenic plants showed significantly higher tolerance to the target pest and performed better when compared to the wild type .The larvae that survived after feeding on the transgenic plants were severely stunted when compared to the larvae that fed on the leaves from the wild type plants in the bioassay Interestingly the efficacy of the chimeric gene appeared similar against the larvae as confirmed by the strong correlation between the percent mortality of the larvae by the plant that is, the average mean percent larval mortality recorded in transgenic plants was 49.6 ± 25.59 (n = 275) compared to that of 3.5 ± 4.73 in non-transgenic wild type plants (n = 13). The„t" test indicated significant differences between the transgenic and non-transgenic plants (t = 12.94; $p < 0.01$). The average mean percent leaf damage recorded in transgenic plants was 34.10 ± 20.53 (n = 275) compared to that of 92.7 ± 3.13 in non-transgenic plants (n = 13) and the difference between the two sets of plants was significant (t = 20.36; p < 0.01 (Table 1). Association between mean percent larval mortality of *H. armigera* and mean percent leaf damage in *cry1X* plants shows (r = -0.596; n = 288; $p < 0.01$) was observed among the putative transgenic plants. This relation followed the equation, $y = -0.4134x + 54.59$ (Figure 7).

Relationship between the insect mortality and the response to ELISA among the transgenic plants

All putative transformants were subjected to both ELISA and detached leaf bioassay against *H. armigera*. However, only those that were positive to ELISA were further tested for the presence of the transgene by attempting to identify the coupled marker *N. phosphor-transferase* gene in the putative transformants. But surprisingly, only 4 T1 plants recorded no larval mortality while 11 plants recorded 10% or less mortality of the *H. armigera* larvae. Similarly, only 47 plants recorded less than 25% larval mortality. As this was unexpected on the basis of the ELISA results, an attempt was made to associate the two parameters to understand the correspondence between the two parameters verified. In order to reduce the ambiguity, 25% larval mortality was

Figure 6. Screening of transgenics KAK-2 T_1 generation plants for *H. armigera resistance. Insecticidal assay with nenate larvae reared for 4 days of H. armigera on leaves from transgenic plant with* cry1X gene (left) and non-transgenic plant (right).

Table 1. Mean percent larval mortality and mean per cent leaf damage observed in *H. armigera* when tested against T_1 generation transgenic plants carrying *cry1X* gene and non-transgenic plants of KAK-2 chickpea variety.

				Helicoverpa armigera	
	n	**% larval mortality**		**% leaf damage**	
		Mean ± SD	**Range**	**Mean ± SD**	**Range**
Transgenic plants	275	49.6± 25.59(44.77)	0-100(0- 90.0)	34.10 ± 20.53(35.73)	5-90.0(12.9-71.57)
Non-transgenic plants	13	3.5 ± 4.73(10.78)	0-15(0-22.79)	92.7 ± 3.13(74.32)	87.5-100(69.3-90.0)
t- test *		12.94		20.36	
P values		*<0.01*		*<0.01*	

*„t" test: two samples assuming unequal variances values in parentheses are arc sine transformed values.

taken as the cut off value to consider a plant as potentially transformed as the mean mortality observed in any wild type plant was 22%. Contingency *Chi* square revealed that the two parameters are not congruent suggesting that it is possible that the expression of the toxin identified by ELISA as is being followed may not fully reflect the activity of the transgene*cry1X* in these putative transgenic plants (λ=0.0034) (Table 2).

Mean percent larval mortality and mean percent leaf damage observed in *H. armigera* in*cry1X* gene incorporated KAK-2 T_1 generation chickpea plants selected for advancement

Based on all the results 43 plants of T_1 generation were selected for further advancement. A range of mortality was observed from 100 to 5% among the plants selected. The average mean percent larval mortality recorded in selected transgenic plants for further advancement was 80.59 ± 24.75 (n = 43) compared to that of 3.5 ± 4.73 in non-transgenic plants (n = 13) 96 h after bioassay initiation. The„t" test indicated significant difference between the T_1 generation transgenic plants selected for further advancement and the non-transgenic plants (t = 15.95; p < 0.01). The extent of mean percent leaf damage caused by *H. armigera* varied from 5.0 to 62.50. The average mean percent leaf damage recorded in selected transgenic plants for further advancement was 22.44 ± 17.52 (n = 43) compared to 92.7 ± 3.13 in non-transgenic plants (n = 13) and the difference was highly significant (t = 20.71; p < 0.01) (Table 3).

Correlation between *cry1X* protein (µg/g of tissue) and mean percent larval mortality of *H. armigera* in T_1 generation KAK-2 chickpea plants

The range of toxic protein level was 0.0 to 10.8 microgram per gram tissue among transgenic T_1

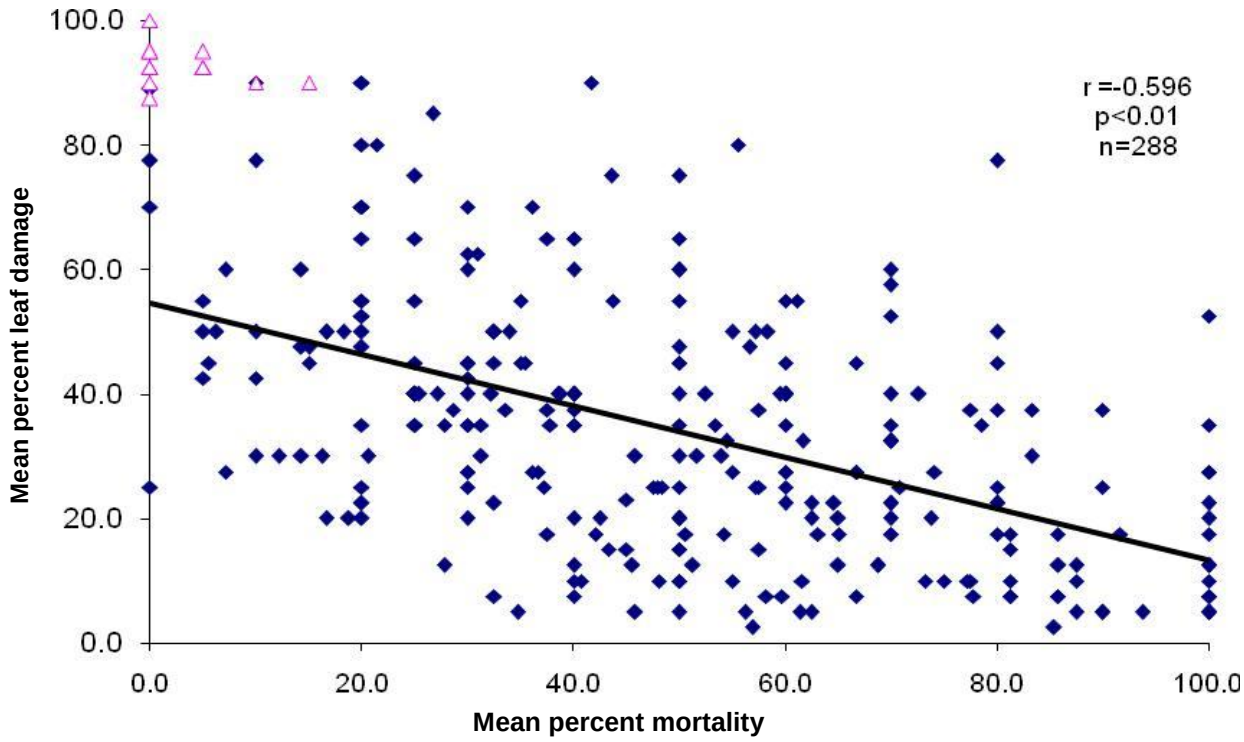


Figure 7. Relation between mean percent larval mortality and mean percent leaf damage of transgenic T_1 generation KAK-2 chickpea plants when tested against *H. armigera* larvae.

Table 2. Relationship between the insect mortality and the response to ELISA among the transgenic plants. Using contingency *Chi* square test.

Chi square test	ELISA		Total
	Number of plants positive for ELISA	Number of plants negative for ELISA	
Plants with >25% larval mortality	189	40	229
Plants with <25% larval mortality	31	15	46
Total	220	55	275

NS: λ>0.05, * λ =0.05, ** λ = 0.01.

Table 3. Mean percent larval mortality and mean per cent leaf damage observed in *H. armigera* when tested against T_1 generation selected transgenic plants carrying *cry1X* gene and non-transgenic plants of KAK-2 chickpea variety.

				Helicoverpa armigera	
	n	**% larval mortality**		**% leaf damage**	
		Mean ± SD	**Range**	**Mean ± SD**	**Range**
Transgenic plants	43	80.59±24.75 (63.86)	5-100(0- 90.0)	22.44± 17.52(28.28)	5-62.5(12.9-52.24)
Non-transgenic plants	13	3.5 ± 4.73(10.78)	0-15(0-22.79)	92.7± 3.13(74.32)	87.5-100(69.3-90.0)
t- test *		15.95		20.7144	
P values		<0.01		<0.01	

*„t" test: two samples assuming unequal variances values in parentheses are arc sine transformed values.

generation KAK-2 chickpea plants. It is generally anticipated that, the larval mortality varies with the cry

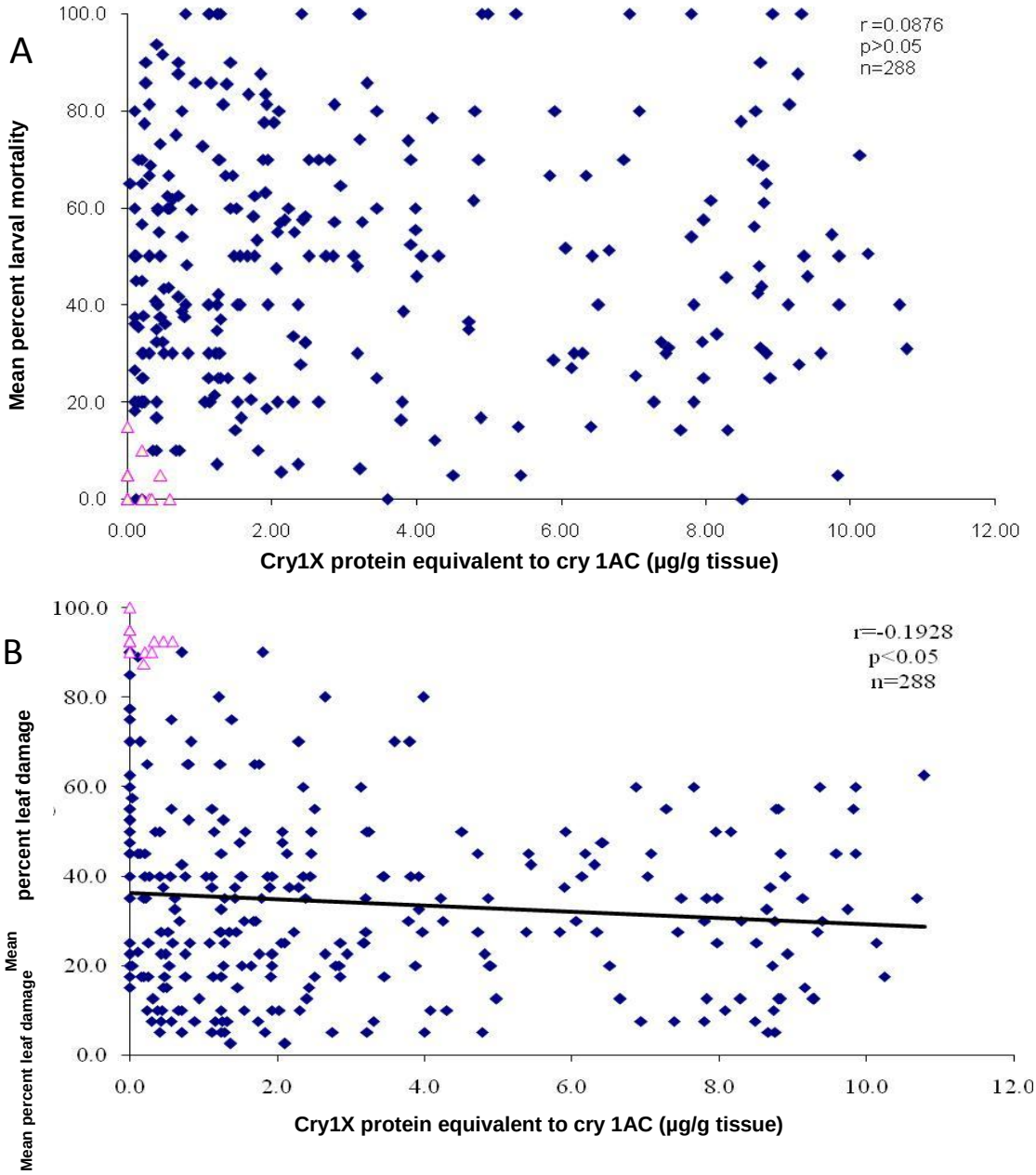


Figure 8. Relation between Cry1X protein expression (µg) and (A) larval mortality of *H. armigera;* (B) mean percent leaf damage caused by *H. armigera* in transgenic T_1 generation KAK-2 chickpea plants.

protein level expressed. This was checked by associating the two parameters for the *cry1X* transgenics in T_1 generation. A non-significant association between *cry1X* protein in a plant (x) and mean percent larval mortality (y) ($r = 0.0876$; $n = 288$; $p < 0.05$) was observed in these plants (Figure 8A). However, plants with higher level of protein expression and high mean larval mortality were also observed. Nevertheless, the results are clearly counter intuitive and are the product of multiple consideration of both ELISA and mortality values in the selection of the plants for further advancement.

Relation between*cry1X* protein (µg/g of tissue) and mean percent leaf damage caused by *H. armigera* in total T_1 generation KAK-2 chickpea plants

As a corollary to the above relationship, one can anticipate similar results for associational studies between *cry1X* protein expression in a plant (x) and mean percent leaf damage (y). This relation was observed to be negative ($r = -0.1928$; $n = 288$; $p < 0.05$). This association therefore clearly complements the previous observation (Figure 8B).

DISCUSSION

Insect pests have major effects on agricultural productivity and food supply. Although the application of insecticides has helped to minimize the impact of insect pests, chemical control entails economic, health and environmental costs. Therefore, the development of new strategies for insect pest control is critical for sustaining agricultural production and improving our environment and health. Insect resistant transgenics provide an exciting option as they are likely to (a) reduce the usage of insecticides for pest management, (b) provide sources of resistance for deployment, which are otherwise unavailable from natural plant sources, and (c) help mitigate further investment on pest management over and above the basic requirements for raising a crop and this factor would be of unmatched importance in improving the productivity of dry lands, if deployed effectively against insect pests of dry land crops. Keeping these factors in view, an attempt was made to explore the possibility of developing insect resistant chickpea over expressing the synthetic *cry1X* gene against *H. armigera.* In essence, the study aimed at understanding the development, evaluation and advancement of constitutively co-expressing *cry1X* gene in transgenic chickpea plant in the kabuli variety KAK-2, along with a kanamycin resistant marker gene, *N. phosphor-transferase. Cry1X* is a synthetic gene comprising the elements of *cry1Aa, cry1Ab, Cry1Ac* and *cry1F*. All these genes are basically active against lepidoptera and have varied efficacy against different taxa. The gene was synthesized by Dr. P. Ananda Kumar, Depatment of Biotechnology, IARI, New Delhi and kindly lent to the University of Agricultural Sciences, Bangalore for the work in the form of a binary vector, pBin-Bt8, cloned in to the strain EHA-105 of *A. tumefaciens*. The chickpea variety KAK-2 was tested for transformation with *cry1X* gene, primarily targeting against the chickpea podborer, *H. armigera*. Transgenic chickpea plants were generated by following an *Agrobacterium* mediated *in planta* transformation protocol for incorporating *cry1X* gene. Procedure involved co-cultivating differentiated embryo of the germinating seeds with *A. tumefasciens* carrying the intended transgene.

In T_0 generation, 44 plants of KAK-2 could be obtained after transformation with *cry1X*. From these, 1060 seeds were obtained for sowing in T_1 generation, which represented the putative transgenics. As the T_0 plants are expected to be chimeric, it is only in the T_1 generation that the true trasformants can be identified. These putative transgenics were then advanced to T1 generation and analyzed adopting multiple evaluation strategies, such as PCR, ELISA, Western blotting and bioassays, for selection of plants for further advancement. Individual PCR studies of selected plants were carried out with both gene specific and *N. phosphotransferase* primers. The PCR positives in subsequent generations provide us the idea of the homozygosity of the transgene in the plants under scrutiny. Percent success of true transformants by PCR analysis showed about 15.63% in the T1 generation. ELISA was conducted based on the antiserum developed for *Cry1Ac* protein. Protein levels were quantified based on standard OD values of the pure protein and expressed as microgram per gram leaf fresh weight.

The maximum cry protein concentration of *cry1X* transgenic plants reached approximately 10.77 µg/g leaf fresh weight in T_1 generation KAK-2 plants. The associational studies between cry protein levels expressed in transgenic plants and mean percent larval mortalities of *Helicoverpa* were observed to be non-significant. Similar trend was also seen between cry protein expression levels and mean percent leaf damage caused by *Helicoverpa.* The western analysis of PCR-positive plants in T_1 generation in KAK-2 plants, revealed the presence of *cry1X* protein in the genome. Bioassays with *Helicoverpa* revealed an average mean percent larval mortality of 49.6 and an average mean leaf damage of 34.10 in T_1 generation KAK-2 putative transformants, where as the corresponding figures for non-transgenic control plants were 3.5 and 92.7%. Under green house conditions, a study was also conducted to ascertain the agronomic parameters and efficacy against *H. armigera* in T_1 generations of putative transgenic KAK-2 variety. The results revealed that in KAK-2, transgenic and non-transgenic plants did not show much difference. There were significant differences in the agronomic traits observed between the transgenic plants and wild types for plant height, number of branches per plant. Whereas non-significant differences were observed for the pod characters such as number of single seeded pods per plant, number of double seeded pods per plant, number of total pods per plant and number of total seeds per plant.

In summary, the study clearly demonstrated potentialities of developing insect resistant transgenics in chickpea. Further, the study also was an example of symbiosis between, biotechnologists and entomologists that helped the progress of the work with a recalcitrant crop such as chickpea, a crop that is not easily amenable for tissue culture. Lastly, the current situation of the transgenic plants developed under this programme, despite being an experimental approach, still provides an opportunity for further test and advancement to reach the field utility level. This is because the bioassays under contained and forced feeding conditions may under estimate the true advantage to be gained under open field conditions.

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