DISCUSSIONS
For effective insect pest management, it is necessary to have basic information on the life cycle of insect pest. In the present investigations, the homogenous culture of potato tuber moth was maintained on potato tubers under the laboratory conditions.

REARING OF POTATO TUBER MOTH AND ITS GENERAL BIOLOGY

Observation on the duration of developmental and adult stages of potato tuber moth reared on potato tubers revealed that the mean duration of the egg incubation is 3.3 ± 0.15 days. The mean duration of the larval, pupal and adult (first to fourth instar) is 16.4 ± 0.30, 6.9 ± 0.31 and 7.1 ± 0.31 days respectively (Table 1). The mean number of the eggs laid by a female moth is 128 ± 9.03 (Table 1). Similar findings were earlier reported by Mukherjee (1949) and Verma (1967). However, Gubbaiah and Thontadarya (1977) reported average incubation period of 5 days both in field and laboratory conditions and average pupal period of 5.6 and 8.5 days in field and laboratory conditions respectively. These workers reported total life period of 20 to 30 days under the field condition and 27 to 40 days under laboratory conditions. Lal and Gupta (1952) reported that the number of eggs laid per female varied from 80 to 150. But, Verma (1967) could not get more than 80 eggs per female. Isahaque (1978) observed that fecundity varied during the different periods of year and it ranged from 50 to 72 during November to December and 95 to 135 during August to September. The variation in
general life span and the progeny produced by potato tuber moth may be due to different climatic conditions prevailing in different regions.

**STANDARDIZATION OF ARTIFICIAL DIET**

In an attempt to standardize artificial diet for the rearing of potato tuber moth under laboratory conditions, three earlier reported artificial diets were tried. The first artificial diet tried in present study was reported by Gleave *et al.* (1998) who used this artificial diet for determining LD$_{50}$ value of *Bacillus thuringiensis* insecticidal crystal protein Cry9Aa2. These workers used Lima bean powder, lactic casein, ascorbic acid and homogenized potato leaves with preservatives and antibiotics. Another diet tried was based on Badegana and Ngameni (2000). These workers used potato flour and vitamin C with antibiotics. The third artificial diet used in the present study was as reported by Singh and Charles (1977).

In the present investigations, it was observed that when larvae of the potato tuber moth were reared on artificial diet described by Gleave *et al.* (1998), the larvae fed initially on this diet but were not able to establish on it and died early in the first instar stage. The larvae could not establish even on the diet described by Badegana and Ngameni (2000) and these fed only on the surface of diet and died in the earlier stages i.e. first and second instars. On both these diets, the mortality could probably be due to compact nature of diets as the larvae were unable to penetrate. However, the larvae of potato tuber moth survived in the Singh and Charles (1977) artificial diet. The successful
establishment of larvae of potato tuber moth on this diet (Singh and Charles, 1977) was possibly due to the coarser fibers of the cellulose, which apparently modified the physical texture of the diet. However, on this diet also, only a few larvae were able to complete their life cycle. The lesser survival of the larvae of potato tuber moth could possibly be due to the reason that the larvae needed more cellulose content for establishment and development.

The modified Singh and Charles (1977) diet was used in the present investigations, as it provided better survival of potato tuber moth larvae. The modification of Singh and Charles (1977) diet was done by increasing cellulose content from 10 g to 13 g and replacing nipagin M with streptomycin and penicillin G (Table 2). Increased cellulose content and wide spectrum antibiotics helped in the rapid establishment of the larvae in the medium and thus a homogenous population could be obtained. The larvae of the potato tuber moth are very delicate and often get drowned in the water film on the surface of this diet. In order to avoid this, artificial diet was dried overnight in the laminar airflow bench.

The basic potato tuber moth culture was maintained on the potato tubers as it is the best natural food for this insect pest. In the experiments, the potato tuber moth larvae were reared on the leaves as it is not possible to take out the second instar from the tubers due to the boring nature of the larvae. Thus, the comparison was done between diet-reared potato tuber moth and those reared on the potato leaves.
In general, the duration of various stages of potato tuber moth was much longer in both treatment-1 (on artificial diet only) and treatment-2 (both on leaves and artificial diet) than on potato leaves (control) (Table 3). Mean duration of first instar larvae was significantly more (4.8 days) on artificial diet in treatment-1 as compared to control (3.2 days). The mean duration of first instar on potato leaves in control (3.2 days) and potato leaves in treatment-2 (3.7 days) was almost same (Table 3). The duration from second to fourth instar larvae was significantly shorter (13.4 days) on potato leaves (control) as compared to artificial diet in treatment-1 (16.8 days) and treatment-2 (17 days). The duration of pupae was significantly shorter (6.6 days) on potato leaves (control) as compared to artificial diet in treatment-1 and treatment-2, in which it was almost same i.e. 8.4 and 8.0 days respectively (Table 3). The duration of adults raised on potato leaves control was significantly shorter (4.3 days) as compared to adults raised on artificial diet in treatment-1 (5.1 days) and treatment-2 (5.3 days). The duration of adults in treatment-1 (5.1 days) and treatment-2 (5.3 days) was almost the same (Table 3).

Singh and Charles (1977) reported egg incubation period of 3 days on potato tubers and 3 days on artificial diet and same was observed in the present studies. These workers observed larval period of 11.8 ±0.2 days, 12.9±0.2 days, 13.2±0.2 days and 13.0±0.2 days on potato tubers, on artificial diet in first generation, second generation and third generation respectively. These workers observed pupal period of 4
to 5 days, 5.9±0.1, 5.5±0.1 and 5.5±0.1 days on potato tubers, on artificial diet in first generation, second generation and third generation respectively. The larval and pupal periods reported in the present studies were longer than those reported by Singh and Charles (1977). The variation in the duration of various stages of potato tuber moth may be due to the differences in the laboratory conditions as Singh and Charles (1977) maintained potato tuber moth culture at 30°C while in present studies, potato tuber moth culture was maintained at 26°C.

Sharaby and Saleh (1985) studied the biology of potato tuber moth by feeding the larvae on different formulations of a semi-artificial diet as well as on potato and observed the efficiency of dry leaves of host plants belonging to family Solanaceae for their efficiency in rearing potato tuber moth. The leaves were mixed with other ingredients of diet. The duration of larval stage reported on diet 1, diet 2 and diet 3 was 12.2±1.62, 6.2±0.35 and 12.8±0.46 days respectively. The duration of pupal stage reported by these workers was 6.2±0.35, 7.4±0.10 and 7.2±0.72 on diet 1, diet 2 and diet 3, respectively. However, the duration of larval and pupal stage was 15.4±1.12 and 6.5±0.52 days respectively on potato tubers (Sharaby and Saleh, 1985).

The larval and pupal periods on the potato tubers observed in present studies are similar to those reported by Sharaby and Saleh (1985). However, the larval and pupal periods on artificial diet in treatment-1 and treatment-2 were longer in present studies as compared to those reported by these workers. The variation in these durations
could possibly be attributed to the differences in the compositions of the diet used and the various nutritional factors of the diets.

Under treatment-2, in which the first instars were initially fed on potato leaves, the duration of first instar was almost same as observed in those fed on potato leaves. The durations of other stages were longer when larvae were transferred to artificial diet. This indicates presence of enhancing nutritional factors in potato leaves, which probably are not present in artificial diet.

The mean percent survival of the different stages of potato tuber moth on artificial diet in treatment-2 (89.2 percent) was almost the same as in control (89.6 percent). However, the mean percent survival of different stages in treatment-1 was significantly less (51.2 percent) as compare to control and treatment-2 (Table 4). The mean percent survival of first instar larvae was significantly higher on potato leaves in control (98.6 percent) as compared to artificial diet in treatment-1 (60.0 percent) and potato leaves in treatment-2 (96.6 percent) (Table 4). The mean percent survival from second to fourth instar larvae was significantly higher on potato leaves in control (93.3 percent) as compared to artificial diet in treatment-1 (54.0 percent) and treatment-2 (91.3 percent) (Table 4). The mean percent survival of pupae was significantly higher on artificial diet in treatment-2 (86.6 percent) as compared to potato leaves in control (84.0 percent) and in treatment-1 (46.4 percent) (Table 4). The mean percent survival of adults was significantly higher (82.6 percent) when fed on potato leaves in control
as compared to artificial diet in treatment-1 (44.0 percent). However, the mean percent survival of adult stage was same in control (82.6 percent) and treatment-2 (82.6 percent) (Table 4).

Singh and Charles (1977) observed 100 percent survival of potato tuber moth (larva to adult) on potato tubers while it was reported as 73.2, 81.9 and 74.2 percent in the first generation, second generation and third generation of potato tuber moth respectively. Sharaby and Saleh (1985) reported 89, 96, 60 and 20 percent moth emergence on potato tubers, diet 1, diet 2 and diet 3 respectively.

In treatment-2, the first instar larvae were initially fed on potato leaves and then transferred to artificial diet. Better survival in treatment-2 may possibly be due to the better establishment of older larvae on the diet. It seems that there is some critical nutritional requirement during first instar stage, which is met by feeding the larvae on potato leaves. It remains to be seen how the supplementation of artificial diet with potato tuber/leaf extracts has effects on bionomics of potato tuber moth.

In the present study 89.2 percent survival was recorded on artificial diet in treatment-2 (Table 4). However, Singh and Charles (1977) observed 73.2 percent survival in first generation. Rearing the first instar larvae on potato leaves and then transferring the second instars on the artificial diet possibly increased the percent survival of larvae in the present investigations.

Female potato tuber moth raised on potato leaves laid significantly more eggs as compared to the females raised on artificial
diet in treatment-1 and treatment-2 (Table 5). Percentage hatch was also significantly more (77.4 percent) in the eggs laid by female potato tuber moth raised on potato leaves as compared to treatment-1 (35.9 percent) (Table 5). Better fecundity and percent hatch in potato tuber moth reared on potato leaves may be attributed to good natural food in the form of potato leaves.

Singh and Charles (1977) observed fecundity of 169±13.6, 95.6±8.4, 100±8.4 and 110±8.3 for the female potato tuber moth raised on potato tubers, artificial diet in first generation, second generation and third generation respectively. Sharaby and Saleh (1985) observed fecundity of 172±16.4, 190.8±13.2, 192.5±9.34 and 42.4±5.16 for the female potato tuber moth reared on potato tubers, diet 1, diet 2 and diet 3 respectively. The fecundity of female potato tuber moth reared on the artificial diet was less as compared to those reported by Singh and Charles (1977) and Sharaby and Saleh (1985).

The present studies indicate that the modified Singh and Charles (1977) diet could be helpful in maintaining potato tuber moth round the year for using them as a food source in mass rearing of natural enemies and parasites in the integrated pest management programs.

STUDIES ON THE EFFICACY OF CRY PROTEINS FOR MANAGEMENT OF POTATO TUBER MOTH

Many insecticidal crystal proteins of \textit{B. thuringiensis} are reported to be toxic to larvae of potato tuber moth e.g. Cry1Aa (Chan \textit{et al.}, 1996), Cry1Ab (Jansen \textit{et al.}, 1995; Chakrabarti \textit{et al.}, 2000), Cry1Ac
(Davidson et al., 2002), Cry 1Aa1 (Mohammed et al., 2000; Douches et al., 2002), and a hybrid Bt toxin, SN19 (Naimov et al., 2003). But the LC50 value of only one insecticidal crystal protein i.e. Cry9Aa2, 80 ng/ml (Gleave et al., 1992) is available in the specificity database of Bt toxins (www.glfc.forestry.ca/bacillus/BTPrints.cfm). Therefore, the present diet and rearing method were used to calculate the LC50 value of these Cry proteins and to find out the most toxic Cry protein against potato tuber moth larvae. Although rearing of potato tuber moth on potato leaves was found to be better in the present studies but this diet seems to be more useful for maintaining the culture of potato tuber moth round the year making available the stages of this insect pest for testing the effects of potential insect control agents as well as for evaluation of insecticidal substances including insecticidal crystal proteins.

The specificity of the isolated Cry proteins was checked on 8 percent SDS-polyacrylamide gel. The protoxin and toxin of Cry1Ab, Cry1Aa, Cry1Ac, Cry1B and Cry fused (Cry1Ab+Cry1B) showed the bands of ~135 kDa and ~ 65 kDa respectively (Fig 9). Earlier, Hofte and Whitley (1989) also reported that size of protoxin of Cry1 type is approximately 130 kDa and the size of toxin is in between 50 to 65 kDa.

LC50 values for the different Cry proteins were calculated against larvae of potato tuber moth by incorporating the various concentrations of these proteins in the modified Singh and Charles diet (1977). The LC50 values calculated for Cry1Aa, Cry1Ab, Cry1Ac, Cry1B and Cry fused were 264.3 ng/ml, 254.7 ng/ml, 276.0 ng/ml, 1933.5 ng/ml and
237.8 ng/ml respectively (Table 6). Cry fused (Cry1Ab+Cry1B) was having the lowest LC$_{50}$ value (237.8 ng/ml), therefore, it was the most toxic cry protein against potato tuber moth (Table 6). The effect of the fused protein on the survival of the potato tuber moth larvae is shown in figs. 10, 11, 12 and 13. Cry1B was having the highest LC$_{50}$ value (1933.5 ng/ml), therefore, it was the least toxic Cry protein against potato tuber moth (Table 6). Gleave et al. (1992) reported the LC$_{50}$ value of 80 ng/ml for Cry9Aa2 against larvae of potato tuber moth.

With the LC$_{50}$ value, additional information on the toxicity of these proteins towards potato tuber moth could be obtained. In the fused gene (cry1Ab+cry1B) pyramiding i.e. fusion of two different cry genes, cry1Ab and cry1B, was done. The fact that different crystal proteins bind to different receptors in the insect gut suggests that the combination of different proteins could retard development of resistance (Gould et al., 1992). With the LC$_{50}$ value calculated in the present studies, it may further be possible to pyramid or fuse two or more toxic proteins for integrated pest management of potato tuber moth. Thus, it could be helpful in delaying the resistance in the transgenic plants.

**AGROBACTERIUM MEDIATED GENETIC TRANSFORMATION OF POTATO**

Genetic transformation is one of the most attractive means of introducing foreign DNA into plants since this technology allows introduction of desired traits to the pre-existing genotype within a short
period as compared to traditional breeding strategies. The *Agrobacterium* mediated transformation is associated with single gene insertion or low copy integration of full length gene (Puterka *et al*., 2002).

The *cry1B-cry1Ab* fused gene used for cloning into binary vector was present in pBluescript II KS⁺ (Fig. 16). The *cry1B-cry1Ab* fused gene in pBluescript II KS⁺ was cut with *NcoI* and *Sall* enzymes. The fragment of 3.8 kb was obtained. The restricted *cry1B-cry1Ab* fused gene fragment was ligated to the binary vector pBinAR containing CaMV 35S promoter and octopine synthase polyA sequence (Fig. 17, 18). The resulting vector was designated as pBinBt6 (Figs. 19, 20). The pBinBt6 vector was transformed into *Agrobacterium* strain EHA 105 by freeze thaw method.

For genetic transformation of potato *Agrobacterium* mediated transformation was used. The internodal cuttings of potato cultivar, Kufri Badshah were used as explants. These explants were kept on preculture medium for two days and then co-cultivated with *Agrobacterium* strain EHA105 containing fused gene. After two days the internodal cuttings were transferred on the selective or regeneration medium containing kanamycin sulphate (100 mg/l) and carbenicillin (200 mg/l). After four weeks, the transgenic shoots started developing (Figs. 21, 22). The shoots thus developed were then transferred to the selective medium containing kamamycin sulphate (100 mg/l) and carbenicillin (200 mg/l) for sometime and then shifted to propagation medium (Fig. 23). Total thirty nine transformants were obtained and out
of these, eight turned yellow or whitish and were, therefore, discarded. Thirty one putative transgenic lines were maintained on the propagation medium. The transformation was carried out under normal tissue culture conditions (26 ± 2°C, 16 hours photoperiod).

In the present investigations, a small but distinct callus phase was observed during initial stages but subsequently with the supplement of zeatin riboside in the regeneration medium, it was found that direct organogenesis resulted in formation of independent transgenic shoots after four weeks (Fig. 23). Multiple shoots formation were seen in some internodes but only one shoot was counted as independent transgenic event. Selection of the transformed shoots was based on the tissues that expressed the selectable marker, nptII gene, which encoded for antibiotic kanamycin sulphate. Transformants that were able to grow on regeneration medium supplemented with kanamycin were selected for further confirmation of the insertion and expression of the gene. Morphologically all putative transgenic plants appeared to be normal and were indistinguishable from the control, Kufri Badshah plant.

Several promoters are available in plant systems based on the place of delivery of the transgenes. Most of the crops are under the transcriptional control of CaMV 35S promoter, which is constitutive in nature (Nagata et al., 1987). cry1Aa, cry1Ab and cry1Ac9 genes directed by CaMV 35S promoter were transferred into the potato plants (Chan et al., 1996; Chakrabarti et al., 2000; Davidson et al., 2002). Jansens et al. (1995) transformed the potato plants using the modified
The internodal stem segments of potato were shown to be more prolific in terms of regeneration as compared to other explants. The use of zeatin riboside as a cytokinin in the culture medium, reduced the duration of internodes on regeneration medium and most importantly, a large number of buds developed without induction of somaclonal variations. Earlier, Beaujean et al. (1998) observed that the use of zeatin riboside in the regeneration medium resulted in considerable reduction of the callus phase and acceleration in transgenic bud formation in the internodal stem segments.

Although, other explants like the leaf discs have been routinely used for transformation but they are prone to high degree of somaclonal variations (Horsch et al., 1985). The leaf disc transformation is accompanied by ethylene production, which can retard the growth of transformed cells if produced in excess. Moreover, leaf discs are prone to injury during their manipulation (De Block, 1988). Tuber disc transformation is generally associated with low somaclonal variations and not all cultivars respond to transformation. Regeneration efficiency is found to be depleted, for tubers stored for more than five months (Dale and Kaija, 1995). However, the internodal explants are reported to
be more resistant and amenable to in vitro conditions (Sangwan et al., 1991). It was found that young explants (4 to 5 weeks) used in the present investigations, were highly receptive to regeneration potential and similar findings have been reported in other crops like *Campanula carpatica* (Sriskandarajah et al., 2004).

In the present studies, for transformation, most of experiments were carried out at ambient temperature of 26°C. Temperature is an important factor affecting the capacity of *Agrobacterium* to transfer DNA to plant cells (Salas et al., 2001) as temperature exceeding 30°C had been demonstrated to impede transformation efficiency (Kudirka et al., 1986). Even, the regulatory Vir A protein of *Agrobacterium tumefaciens* is temperature dependent and temperature above 32°C was correlated with no transformation (Jin et al., 1993).

In the present investigations, for effective *Agrobacterium* mediated transformation, the antibiotics were used to control the bacterial overgrowth, without inhibiting the regeneration efficiency of plant cells. A sufficient concentration of the kanamycin is required to suppress proliferation of non-transformed cells. In the present investigations, it was found that 100 mg/liter concentration of kanamycin sulphate is suitable for recovery of transformed cells.

**BIOASSAY AND MOLECULAR ANALYSIS OF TRANSGENIC POTATO LINES**

A common approach to identify the transformed shoots is to use amplification of the selectable marker gene during early developmental
stages through polymerase chain reaction. Transgenic nature of the putative transformants was screened through polymerase chain reaction amplification with nptII primers. All thirty one putative transgenic lines showed positive results for nptII marker gene with the distinct band of 700 base pairs (Fig. 24).

The transgenic lines were further amplified with the cry1Ab gene primers that resulted in the positive amplification of 540 base pairs in all transgenic lines, thus confirming the insertion of fused gene in the transgenic plants (Fig. 25). Similarly, the transgenic lines were amplified with the cry1B gene primers that resulted in the positive amplification of 800 base pairs, confirming the insertion of fused gene in the transgenic plants (Fig. 26).

To determine the varying level of expression in transgenic lines, total RNA of transgenic plants was isolated and reverse transcriptase polymerase reaction was carried out. All transgenic lines showed the positive amplification with cry1Ab and cry1B gene primers showing bands of 540 and 800 base pairs respectively (Figs. 27, 28). This confirmed the expression of fused gene in the transgenic plants.

On the basis of these results, eleven lines were selected for Southern hybridization to check the number of copies of fused gene in the transgenic plants. One copy of gene was present in eight transgenic lines (Fig. 30). Although Agrobacterium mediated genetic transformation results in low copy insertion as compared to other methods such as particle bombardment method that results in multiple copies, in three
transgenic lines, two numbers of copy genes were present (Fig. 30). The multiple copies of gene could result in the inactivation of the transgene, thereby resulting in the post-transcriptional gene silencing.

Evaluation of these transgenic lines against the potato tuber moth was done under the tissue culture conditions as well as in the glass house. The bioassay of the all transgenic lines against potato tuber moth was done under the normal tissue culture conditions. All transgenic lines showed significantly less mean percent survival as compared to non-transformed Kufri Badshah (Table 7). The transgenic line KBdBte 29 showed zero percent mean percent survival of larvae of potato tuber moth, indicating that it is the most resistant against larvae of potato tuber moth. The transgenic lines which showed mean percentage survival of the larvae of potato tuber moth in between 0 to 25 percent under tissue culture conditions were selected for further bioassay under glass house conditions (Table 7).

The transgenic lines showed significantly lesser survival of larvae of potato tuber moth as compared to non-transgenic plants. This indicates the resistance against larvae of potato tuber moth in these transgenic lines (Table 8). Only five lines showed the percentage survival in between 25 to 50 percent (Table 8). The more survival of the potato tuber moth larvae could be due to less expression of the fused gene in the transgenic plants. The mean weight of larvae fed on the transgenic lines was significantly less as compared to the non transformed Kufri Badshah. This may be due to the inhibition of feeding
of larvae of potato tuber moth by the fused gene. The mean percentage survival of potato tuber moth larvae ranged from 30.0 to 86.6 percent in the transgenic lines (Table 8). The mean weight of larvae fed on the leaves of transgenic lines ranged from 0.2 to 1.6 mg in the transgenic lines (Table 8). The present studies are in accordance with the earlier reports of other workers (Jansens et al., 1995; Lagnaoui et al. 2000).

The results of the leaf bioassay observed in the present studies were in accordance with those reported by Jansens et al. (1995), who introduced truncated version of cry1Ab gene into potato cultivars Kennebec, Bintje and Yesmina. These workers reported significantly less weight of the larvae of potato tuber moth fed on the transgenic lines expressing cry1Ab gene as compared to the control. The percentage mortality of the potato tuber moth larvae ranged from 35 to 100 percent in the transgenic lines.

Similar results were observed by other workers in the transgenic lines expressing Bt-cry5 gene. Tailor et al. (1992) modified a Bt-cry5 toxin gene, with activity against both lepidopterans and coleopterans to increase the level of expression of this gene in the transgenic plants. Douches et al. (1998) introduced this gene into potato to obtain resistance implants against potato tuber moth. Detached leaf bioassays with Bt-cry5 potato lines demonstrated a high level of Bt-Cry5 protein in the leaves and upto 96 percent mortality of larvae of potato tuber moth was reported by Westedt et al. (1998) and Li et al. (1999).
Lagnaoui et al. (2000) reported significantly higher mortality of larvae of potato tuber moth on the leaves of transgenic potato lines expressing Bt-cry5 gene (cry1la1) as compared to the control. The mortality of the larvae in transgenic lines ranged from 80 to 90 percent. Naimov et al. (2003) had observed 100 percent mortality of potato tuber moth larvae on the leaf of transgenic lines expressing SN19 hybrid gene during leaf bioassay.

In the present investigations, it was found that mean percentage survival of larvae of potato tuber moth during tuber bioassay was significantly lesser in all twenty one transgenic lines (Table 9). The mean percentage survival of potato tuber moth ranged from 16.6 to 80.0 percent in the transgenic lines (Table 9). Only in three transgenic lines, the percentage survival of the potato tuber moth was more than 75 percent. Possibly, the higher percent survival of larvae of potato tuber moth was due to the low expression of the fused gene in these transgenic lines. Earlier, Jansens et al. (1995) reported almost 100 percent mortality of potato tuber moth on the stored potato tubers of transgenic lines expressing Cry1Ab gene indicating high expression of this gene in the tubers. Similarly, Lagnaoui et al. (2000) observed high levels of resistance (100 percent larval mortality) in all Spunta transgenic lines in tubers. Chakrabarti et al. (2000) also reported the significant degree of resistance in the potato plants expressing cry1Ab gene under the control of CaMV 35S promoter against potato tuber moth.
Mohammed et al. (2000) assayed the newly harvested and stored tubers of transgenic lines of potato cultivars Lemhi Russet, Atlantic and Spunta expressing Bt-cry5 gene (cry1a1) against potato tuber moth larvae. In tuber bioassay using stored tubers of Bt-cry5-Lemhi Russet transgenic lines and Bt-cry5-Atlantic potato lines, these co-workers observed up to 100 percent mortality of first instar. Lowest mortality was observed in newly harvested tubers of Bt-cry5-Atlantic potato lines (47.1 to 67.6 percent). Potato tuber moth larvae mortality was 100 percent in the Bt-cry5-Spunta potato lines that was transformed with Bt-cry5 gene controlled by CaMV 35S promoter (pBIMLS vector) and in two of three lines transformed with Bt-cry5 gene controlled by the Gelvin Super promoter (pBMIL1 vector). These co-workers observed lowest potato tuber moth mortality in the transgenic lines expressing Bt-cry5 gene controlled by patatin promoter (pBMIL2 vector). Potato tuber moth mortality in the Atlantic transgenic lines ranged from 47.1 to 67.6 percent. In all the cases, potato tuber moth mortality in fresh tubers of all Atlantic transgenic lines was lower than the mortality in stored tubers. Mortality on the two lines with the constitutive Gelvin super promoter was 100 percent, mortality on three lines with the constitutive 35S promoter was 66.9 to 100 percent, whereas mortality in the two Bt-cry5 lines with the patatin promoter was very low (25.6 and 31.1 percent mortality). The various levels of mortality in the transgenic lines indicate that the different promoters used affected the expression of the gene in these lines. The expression of the gene was high in the transgenic lines
expressing gene controlled with CaMV 35S promoter and less in transgenic lines expressing gene controlled with patatin promoter.

In the present investigations, mean number of galleries with smaller surface area was more in the transgenic lines as compared to control Kufri Badshah, whereas mean number of galleries with the larger surface area was more in the control Kufri Badshah as compared to transgenic lines (Table 10). The mean number of class I type galleries made by the larvae of potato tuber moth was significantly different in transgenic lines as compared to non-transformed Kufri Badshah except KBdBt6 22 (Table 11). The mean number of class II type galleries made by the larvae of potato tuber moth was significantly different in the transgenic lines as compared to non-transformed Kufri Badshah except KBdBt6 12, KBdBt6 27, KBdBt6 29 and KBdBt6 32 which were similar to control (Table 11). The mean number of class III type galleries made by larvae of potato tuber moth was significantly different in the transgenic lines as compared to non-transformed Kufri Badshah except KBdBt6 17, KBdBt6 27 and KBdBt6 40 (Table 11). The mean number of class VI, V and class VI type galleries made by the larvae of potato tuber moth was significantly different in all transgenic lines as compared to non-transformed Kufri Badshah (Table 11).

Galleries with smaller surface area were observed more in the transgenic lines as compared to control (non-transformed Kufri Badshah), whereas the number of galleries with the larger surface area was more in the control (non-transformed Kufri Badshah) as compared
to transgenic lines (Figs. 40, 41, 42, 43, 44, 45, 46 and 47). Jansens et al. (1995) reported similar findings in the green house trial of the transgenic lines. The number of tunnels with the 0 to 5 mm$^2$ was significantly larger in transgenic lines as compared to control. This may be due the reason that sub-lethal concentration of fused gene inhibited feeding of larvae that did not die but recovered and fed again on another place on the leaf resulting in the larger leaf injuries.

Beuning et al. (2001) made minor modification to the nucleotide sequence of truncated cry1Ac9 to produce cry1Ac9$^A$ (one nucleotide change) and cry1Ac9$^B$ (seven nucleotide change). These derivative genes under the control of the CaMV 35S promoter were transformed into Nicotiana tobacum. These co-workers observed lower larval growth, development and survival of potato tuber moth larvae on transgenic lines expressing cry1Ac9$^B$ as compared to control (non-transgenic plants).

The present investigations show that the Kufri Badshah transgenic lines expressing the fused gene (cry1Ab+cry1B) are having various level of resistance against potato tuber moth. Similar findings were reported earlier by other workers (Jansens et al., 1995; Chakrabarti et al., 2000; Lagnaoui et al., 2000; Mohammed et al., 2000 and Naimov et al., 2003). Although, the expression of the fused gene in transgenic plants was less, but the transgenic lines provided significant degree of resistance as compared to the non-transgenic Kufri Badshah. Such potato tuber moth resistant lines should further be evaluated in the field trials and storage conditions.
The transgenic plants expressing the *cry* genes are a powerful tool in integrated pest management program. The expression of fused gene in potato tuber will provide seed producers and growers a tool to reduce potato tuber moth damage to the crop in field and storage. So in the integrated pest management programme, the use of host plant resistance to manage potato tuber moth will greatly reduce the use of pesticides.