EVALUATION OF BIOLOGICAL ACTIVITY OF *Bacillus thuringiensis*, SOYBEAN TRYPSIN INHIBITOR AND PLANT LECTINS AGAINST THE LEGUME POD BORER, *Helicoverpa armigera*

THESIS

Submitted to the
JAWAHARLAL NEHRU KRISHI VISHWA VIDYALAYA, JABALPUR
in partial fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE
IN
AGRICULTURE
(ENTOMOLOGY)

By

SONALI SHUKLA

DEPARTMENT OF ENTOMOLOGY
COLLEGE OF AGRICULTURE
JAWAHARLAL NEHRU KRISHI
VISHWA VIDYALAYA
JABALPUR, M.P. 482004

ENTOMOLOGY UNIT
GENETIC RESOURCES AND
ENHANCEMENT PROGRAM
ICRISAT, PATANCHERU,
ANDHRA PRADESH – 502324

2000

DEDICATED

TO MY

BELOVED PARENTS

CERTIFICATE

This is to certify that the thesis entilted, **"Evaluation of biological activity of *Bacillus thuringiensis*, soybean trypsin inhibitor and plant lectins against the legume pod borer, *Helicoverpa armigera*"** submitted in partial fulfilment of the requirements for the degree of **Master of Science** of the Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, is a record of the bonafide research work carried out by **Ms. Sonali Shukla** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

S. Shrivastava
17/5/2001

Dr. Shail Shrivastava
Chairman of the Advisory Committee

Thesis approved by the Student Advisory Committee

Chairman:
Dr. Shail Shrivastava
Scientist
Department of Entomology,
College of Agriculture, Jabalpur M.P., 482 004

S. Shrivastava
17-5-2001

Members
Dr. H. C. Sharma
Senior Scientist (Entomology)
ICRISAT, Patancheru, A.P. 502 324

Mrs. A. Moitra
Junior Scientist
Department of Entomology,
College of Agriculture, Jabalpur M.P., 482 004

Dr. U. Goswami
Scientist
Dept. of Statistics and Mathematics,
College of Agriculture, Jabalpur M.P., 482 004

Dr. N. G. Mitra
Scientist
Department of Entomology,
College of Agriculture, Jabalpur M.P., 482 004

CERTIFICATE – II

This is to certify that the thesis entitled " **Evaluation of biological activity of *Bacillus thuringiensis*, soybean trypsin inhibitor and plant lectins against the legume pod borer, *Helicoverpa armigera***" submitted by Ms. Sonali Shukla to Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, in partial fulfillment of the requirements for the degree of M. Sc. (Ag.) in the Department of Entomology has after evaluation, been approved by the external examiner and by the students Advisory Committee after an oral examination on the same.

Jabalpur 17.5.2001

Dated : Dr. S. Shrivastava
Chairman of Advisory Committee

Members of the Advisory Committee

Dr. S. Shrivastava (Chairman) 17.5.2001

Dr. H. C. Sharma (Member) .....

Mrs A. Moitra (Member) .....

Dr. U. Goswami .....

Dr. N. G. Mitra .....

HEAD OF THE DEPARTMENT ..... Head Department of Entomology J. N. K. V. Jabalpur

DIRECTOR OF INSTRUCTIONS .....

Director of Instruction
J.N.Krishi Vishwa Vidyalaya
JABALPUR (M.P.)

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my deep sense of gratitude and sincere thanks to ***Dr. S. Shrivastava****, Scientist, Department of Entomology, College of Agriculture, Jabalpur, and Chairman of the Advisory Committee for her stimulating guidance, patient appraisals and the keen interest she has taken to give me the right perspective of research. I shall gratefully memorise the invaluable suggestions and scholarly advises offered by her.*

Words seem inadequate in expressing the respect and thankfulness to ***Dr. H. C. Sharma****, Senior Scientist, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, and co-chairperson of the Advisory Committee. It is a great privilege to have been his student and be benefitted by his immensely valuable and exemplary guidance. His continuous involvement, well timed suggestions, continuous care and constant encouragement during the course of study enabled me in bringing out the thesis into the presentable form to the best of my ability. I have immense pleasure in registering my profound regard, glorious appreciation, heartfelt acknowledgements and deepest admiration for his meticulous guidance.*

I express my profound thankfulness and gratitude to ***Mrs. A. Moitra****, Junior Scientist, Department of Entomology, College of Agriculture, Jabalpur, and member of the Advisory Committee for her encouragement and precious suggestions.*

I acknowledge with thanks, the help and counsel extended by ***Dr. N. G. Mitra****, Scientist, Department of Entomology, College of Agriculture, Jabalpur, for his helpful suggestions.*

I am thankful to ***Dr. U. Goswami****, Scientist, Department of Statistics, College of Agriculture, Jabalpur, and member of the Advisory Committee for his support and encouragement during the progress of work.*

I am thankful to ***Dr. S. M. Vaishampayan****, Professor and Head, the* ***staff of Department of Entomology,*** *College of Agriculture, Jabalpur for their*

encouragement and help.

I express my special thanks to ***Dr. K. K. Sharma*** *and* ***Dr. V. Anjaiah*** *for their generous support and helpful suggestions.*

I am grateful to ***Mr. Venkateshwar Rao*** *for his co-operation and precious suggestions during the analysis of data.*

I appreciate and thank for the assistance and co-operation extended to me by, ***Mr. G. Pampapathy, Mr. Madhusudhan Reddy, Mr. Raja Rao, Mr. N .V. S. Chandra*** *and all the other* ***staff of Entomology Unit*** *throughout my research work at ICRISAT.*

I would like to thank ***Dr. C. L. L. Gowda****, Training and Fellowship program, ICRISAT for permitting me to avail the research facilities at ICRISAT.*

I am highly thankful to ***Mr. B. U. Singh*** *who went out of his way to help me in executing my experiments.*

I am in dearth of words to express my unboundful gratitude, love and affection to my parents ***Mr. Girish Shukla*** *and* ***Mrs. Manju Shukla,*** *who have been a constant source of inspiration and encouragement at each and every step of my career. I would like to express my affection for my sister* ***Manali*** *who shared both the joys and the sorrows with me.*

I affectionately thank all my friends for the valuable and moral support and help. Particularly having ***Sonam, Priya*** *and* ***Anitha*** *around has meant more than I can express.*

For the unboundful grace and abundant blessings I am eternally indebted to ***The Almighty.***

Shukla

(Sonali Shukla)

LIST OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

INTRODUCTION

Cotton bollworm / legume pod borer, *Helicoverpa (Heliothis) armigera* (Hubner), is one of the most destructive pest of field crops. It is widely distributed from the Cape Verde Islands in the Atlantic Ocean, through Africa, Asia, and Australia to the South Pacific islands, and from southern Europe to New Zealand (Reed and Pawar, 1982). It is a polyphagous pest and has been recorded feeding on 181 cultivated and uncultivated plant species belonging to 45 families (40 dicots and 5 monocots) (Manjunath *et al.*, 1989). However, most serious losses have been recorded in crops such as pigeonpea, chickpea, tomato, cotton, sorghum, pearl millet, peas, and groundnut.

The productivity of pigeonpea (Singh *et al.*, 1990) and chickpea (Reed *et al.*, 1987) crops is drastically affected by *H. armigera*, which causes substantial damage and yield losses every year. Annual losses due to *H. armigera* in chickpea and pigeonpea have been estimated to exceed US $ 600 million (ICRISAT, 1992). In pigeonpea alone, it is estimated to cause yield losses equivalent to US $ 317 million annually (Shanower *et al.*, 1999).

Chemical control is one of the effective methods of reducing *H. armigera* damage, but total reliance on insecticides has not been helpful in managing this pest effectively. There are several reports of this pest developing resistance to commonly used insecticides, and failure of insecticides to control the pest under field conditions (Manjunath *et al.*, 1989). The indiscriminate use of pesticides has also led to adverse effects on the beneficial organisms, emergence of secondary pest problems, and contamination of food and food products with insecticide residues.

This situation has led the scientists to develop alternative pest control strategies, which are devoid of these undesirable effects. One of the alternative approaches for *Helicoverpa* control is the development of plants resistant to insect attack. Such plants can be developed by insertion and expression of insect resistance genes into crop plants through genetic

engineering. Genetic engineering of crop plants for insect resistance has many advantages; which include decreased chemical usage, environment-friendly nature, season-long protection independent of weather conditions, effective control of burrowing insects difficult to reach with insecticidal sprays, and control at all stages of insect development.

Different approaches to obtain insect-resistant plants are presently being explored, such as the use of δ- endotoxins coding sequences originating from the bacterium, *Bacillus thuringiensis* (L.), and plant-derived genes such as those encoding enzyme inhibitors or lectins. The greatest research effort in developing pest-resistant transgenic crops has gone into expression of *Bacillus thuringiensis* (Bt) toxins in plants. Their use in cotton against bollworm (*Heliothis virescens* F.) (Perlak *et al.*, 1990), tobacco budworm (*Helicoverpa zea* Boddie) (Hoffmann *et al.*, 1992) and pink bollworm (*Pectinophora gossypiella* Saunders) (Wilson *et al.*, 1992) and in corn against the European corn borer (*Ostrinia nubilalis* Hubner) (Armstrong *et al.*, 1995), has lead to a considerable reduction in insecticide use (James, 1997). The first transgenic tobacco and tomato plants expressing the Bt toxins at levels insecticidal to lepidopteran insects were obtained in 1987 (Barton *et al.*, 1987; Fischhoff *et al.*, 1987; Vaeck *et al.*, 1987). Bt genes have now been introduced into and expressed in a wide range of crop species, including tobacco (Barton *et al.*, 1987, Vaeck *et al.*, 1987, Perlak *et al.*, 1991, Williams *et al.*, 1993, Strizhov *et al.*, 1996); tomato (Fischoff *et al.*, 1987); cotton (Perlak *et al.*, 1990); rice (Fujimoto *et al.*, 1993, Wunn *et al.*, 1996); potato (Peferoen, 1992, Perlak *et al.*, 1993); maize (Koziel *et al.*, 1993); soybean (Stewart *et al.*, 1996a); canola (Stewart *et al.*, 1996b); poplar (McCown *et al.*, 1991, Cornu *et al.*, 1996) and alfalfa (Strizhov *et al.*, 1996).

Another strategy that is similar to using Bt derived toxins, is the utilization of plant-derived inhibitors of proteins. Protease inhibitor proteins are among the defensive chemicals in plant tissues that are produced in response to insect attack. Wound-inducible proteinase inhibitors have been clearly shown

to be involved in plant defense (Ryan, 1990). This involvement of proteinase inhibitors in natural defense by plants has lead towards enhancing plant defense through genetic engineering. Genes encoding members of various serine protease inhibitors (SPIs) have been cloned and introduced into transgenic plants. The first report on the use of a plant-derived insect control protein gene in transgenic plants came with the expression (from the CaMV35S gene promoter) of a gene for cowpea trypsin inhibitor in tobacco (Hilder *et al.*, 1987). These transformed tobacco plants have resulted in resistance to feeding by the tobacco budworm, *H. virescens*. Similar results were shown in a field trail in the USA against corn earworm *(H. zea)* (Hoffmann *et al.*, 1992). Transgenic tobacco has also been shown to enhance protection against *Spodoptera litura* (Fab.) (Yeh *et al.*, 1997) and *Manduca sexta* L. (Johnson *et al.*, 1989).

Another group of compounds, which can be exploited to impart resistance to insects in crop plants, is plant lectins. Lectins are unique proteins with a highly specific carbohydrate (glyco-conjugates) binding activity. They have been isolated from various plant tissues, with seeds being the richest source (Etzler, 1986). They play an important role in the plant's defence against insect pests and have been found to be toxic to viruses, bacteria, fungi, insects and higher animals. Lectins from snowdrop, pea, wheat, rice, castor, soybean, mungbean, garlic, sweet potato, tobacco, chickpea and groundnut have been isolated and characterized. Their effect on the survival and development of insect pests belonging to different insect orders has been studied by several workers in the past (Shukle and Murdock, 1983; Czapla and Lang, 1990; Habibi *et al.*, 1993; Powell *et al.*, 1993; Gatehouse *et al.*, 1995; Powell *et al.*, 1995).

In the present studies, we evaluated Bt, SBTI and plant lectins for their biological efficacy against the legume pod borer *Helicoverpa armigera*. Following were the specific objectives of the present investigation:

1. To evaluate antifeedant activity of Bt and soybean trypsin inhibitor (SBTI) genes against *H. armigera* under dual-choice and no-choice conditions.
2. To evaluate the effect of SBTI and plant lectins on the survival and development of *H. armigera*.
3. To evaluate the effect of Bt and SBTI genes on consumption and utilization of food by *H. armigera*.
4. To evaluate the effect of Bt and SBTI transformed plants on the biology of *H. armigera*.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Helicoverpa armigera (Hubner) is a pest of worldwide agricultural importance. It feeds on a wide range of wild and cultivated host plants. The larvae, particularly the later instars, feed upon the reproductive parts of the plant. In India, it is a dominant pest on cotton, pigeonpea, and chickpea. On pigeonpea and chickpea, it commonly destroys more than half of the grain yield.

The biological characteristics such as high fecundity, extensive polyphagy, strong flying ability, and a facultative diapause contribute to the devastating pest status of *H. armigera* (Fitt, 1989). The ability to feed on various plants enables *H. armigera* populations to develop continuously during the entire cropping season (Bhatnagar *et al.*, 1982).

Biology

The legume pod borer females lay eggs singly, on the upper surface of the leaves along the midrib, flowers, pods and stems. The number of eggs per female ranges from 387 to 1364 on different host plants (Dhandapani and Subramaniam, 1980). The eggs are white and nearly spherical when freshly laid, and darken with age. Eggs hatch in 2 to 5 days. Larval duration varies from 8 to 28 days (Singh and Singh, 1975), and there are 5 to 7 larval instars, which vary with temperature and the host plant. Pupation takes place in the soil, and the adults emerge in 7 to 10 days. One generation can be completed in just over 4 weeks under favorable conditions. There are several generations in a year. The number of generations vary according to agro-climatic conditions. It passes through four generations in Punjab (Singh and Singh 1975), seven to eight generation in Andhra Pradesh (Bhatnagar, 1980), and five generations in Uttar Pradesh (Tripathi, 1985).

Nature of Damage

The young larvae of *H. armigera* feed by scraping green tissues and wanders about nibbling various parts of the plant until it finds a flowerbud or flower, a bud will be hollowed out, leaving an empty shell. In pigeonpea and chickpea, the older larvae chew voraciously into buds, flowers, and pods, leaving characteristic round holes. In cotton, older larvae feed on buds and young bolls and habitually feed with only the front portion of its body inside the hole it has made. Cotton buds and bolls that have been attacked by *Helicoverpa* thus commonly show an accumulation of larval faeces between the surface and the enclosing bracteoles.

Crop losses

The estimated losses due to *H. armigera* vary in different countries viz., US $ 600 million in chickpea and pigeonpea per annum in semi-arid tropics (ICRISAT, 1992); A$16 million in 1979 (Alcock and Twine, 1981), A$23.5 million (Wilson, 1982), and A$25 million annually in Australia (Twine, 1989). In India, crop losses in the pulses, chickpea and pigeonpea were estimated at over $ 300 million per annum (Reed and Pawar, 1982). In recent years, the chemical resistance problem has increased, resulting in estimated crop losses due to this pest in India in 1996-1997 reaching 158 million US $ (Russell, 1999).

Plant genetic engineering

Recent advances in tissue culture and molecular biology have made it possible to introduce foreign genes into crop plants from diverse sources and produce transgenic plants with new genetic properties. Genetic manipulation

number of Bt based products are available for the control of agricultural pests. The worldwide insecticidal sales are estimated to be in range of $6 billion, and the total sales of Bt are estimated to be in the range of $100 million (Lambert and Peferoen, 1992).

The earliest commercial production of Bt began in France in 1938, under the trade name Sporeine. During the 1960s, several formulations of Bt were manufactured in the United States, Soviet Union, France, and Germany; with various degrees of commercial success. A major step forward in the commercial success of Bt was the isolation of HD-1, a strain which proved to be highly potent and which still forms the basis of some today's commercial insecticides. Initially, it was believed that Bt was active only against Lepidoptera. In 1977, Goldberg and Margalit isolated a *B. thuringiensis* subsp.*israelensis*, from a mosquito-breeding pond. This subspecies is highly toxic to mosquito and blackfly larvae. In 1983, Krieg and coworkers isolated *B. thuringiensis* subsp., *tenebrionis*, from dead mealworm pupae, which is highly effective against elm leaf beetle (*Agelastica alni*) and Colorado potato beetle (*Leptinotarsa decemlineata Say*) larvae. These finding lead to the conclusion that Bt can be used against other economically important insects.

Bt formulations have been used in the field for the past 40 years. Despite many advantages, the use of Bt-based insecticides is constrained due to the high cost of production and poor persistence under field conditions. Therefore, it was felt that expression of the toxin genes in transgenic plants might increase their utility in insect control. The approach involving the transfer and expression of *B. thuringiensis* toxin-encoding genes into plants has attracted much attention The ICP (insecticidal crystal protein) genes can be transferred by using various transformation systems (e.g, theTi-plasmid-mediated plant transformation system from *Agrobacterium tumefaciens*). Pest resistance lines have been developed in cotton, poplar (*Populus* spp.), potato, rice and maize through the introduction of genes encoding the insecticidal proteins of *B. thuringiensis*.

The toxin genes were earlier classified into four types, based on protein structural homologies and host range (Hoftey and Whiteley, 1989). Cry I genes, active against lepidoptera encode proteins of 130 kDa; Cry II genes, active against lepidopteran and dipteran larvae, encode proteins of 70 kDa, and Cry III genes active against coleopteran larvae, encode proteins of 70 kDa. Cry IV genes are specific to the dipteran larvae. The system was further extended to include type V genes that encode for proteins effective against lepidopteran and coleopteran larvae (Tailor *et al.*, 1992).

Mechanism of Action

The target organ for Bt toxins is the insect midgut. In general terms, upon ingestion, crystal proteins are dissolved from the crystals and are proteolytically activated to a trypsin resistant core fragment. This protein passes through the pores in the peritrophic membrane and binds to a membrane protein in the brush border of the midgut epithelial cells and inserts into the membrane. This insertion leads to the formation of pores and lysis of the cells.

When Bt crystals are ingested by insects, the crystal proteins are dissolved from the crystal. The pH in the gut of lepidopteran larvae varies between 9 and 12 and lepidopteran-specific crystal bodies can only be solubilized above pH 9.5 (Knowles and Dow, 1993). On getting solubilised in midgut, the crystalline bodies release the proteins called δ-endotoxins. The toxin portion is derived from the N-terminal half of the protoxin, while the C-terminal portion is involved in the formation of parasporal inclusion bodies and is usually hydrolysed into small peptides (Choma *et al.*, 1990). The activated toxin can be divided into three structural regions : a N-terminal region, the toxic domain (amino acids 1-279), consisting of several conserved hydrophobic regions, a conserved C-terminal region (amino acids 461-695), and a variable region (amino acids 280-460) (Aronson *et al.*, 1986; Choma

and Kaplan, 1990; Hofte and Whitley, 1989). The N-terminal region has a significant role in penetrating the peritrophic membrane, while the C-terminal region and the highly variable region are considered important in toxin specificity by coding for open β-sheets that bind to glycoprotein receptors in the midgut (Choma and Kaplan, 1990; Convents *et al.*, 1990). The protoxins are activated by gut proteases, which typically cleave some 500 amino acids from the C terminus of 130 kDa protoxins and 28 amino acids from the N terminus, leaving a 65 to 55 kDa protease-resistant active core comprising the N-terminal half of the protoxin (Hoftey and Whitley, 1989).

Brush border membrane veicles (BBMV) have been identified as the primary binding site for several insect species (Lee *et al.*, 1992). The active toxins bind to the specific receptors located on the apical brush border membrane of the columnar cells. There may be many toxin binding protein receptors, and some have been identified as 12 to 180 kDa glycoproteins (Garczynski *et al.*, 1991; Knowles *et al.*, 1991; Oddou *et al.*, 1991). After binding to the receptor, the toxin inserts irreversibly into the plasma membrane of the cell leading to lesion formation. There is a positive correlation between toxin activity and ability to bind BBMV (Gill *et al.*, 1992), and the toxicity is correlated with receptor number than receptor affinity (Van Rie *et al.*, 1989).

After binding to the midgut epithelial cells, the α-helices can penetrate the apical membrane to form an ion channel (Knowles and Dow, 1993). The formation of toxin induced pores in the columnar cell apical membrane allows rapid fluxes of ions. The pores are K^+ selective (Sacchi *et al.*, 1986), permeable to cations (Wolfersberger, 1989), permeable to anions (Hendrickx *et al.*, 1989), or permeable to solutes such as sucrose, irrespective of the charge (Schwartz *et al.*, 1991). Carroll and Ellar (1993) observed that midgut permeability in the presence of Cry 1A(c) was altered for cations, anions, neutral solutes and water. Knowles and Dow (1993) suggested that Bt toxins lead to cessation of K^+ pump that leads to swelling of columnar cells and

osmotic lysis. The disruption of gut integrity leads to death of the insect through starvation or septicema.

Bt - Transgenic Crop Plants

The first results concerning the transfer of *B. thuringiensis* genes in tobacco and tomato were published in 1987. A Beligan biotechnology company, Plant Genetic System, reported the first successful use of the technology in 1987 (Vaeck *et al.*, 1987). They developed tobacco plants using genes from *B. thuringiensis*. These plants produced endotoxins, which killed the first instars of *Manduca sexta* larvae. Insects placed on the leaves of the plants displayed the same response as insects placed on leaves sprayed with commercial *B. thuringiensis* products feeding suppression after 18 hours, and death within 3 days. Levels of the endotoxin as low as 30 mg per gram of leaf protein provided complete protection against *M. sexta* neonates, and the production of endotoxin was shown to be inherited a simple dominant character. Since then, *B. thuringiensis* genes have been transferred to a number of other crop species such as cotton, rice, and maize with Lepidopterans as the main targets.

Adamczyk *et al.* (1998) studied the survival and development of fall armyworm, *Spodoptera frugiperda J.E. Smith*, on leaves and bolls of the normal and the Cry1A(c) transformed cotton plants and observed that their was no significant difference in larval survival and the number of larvae that pupated and eclosed as adults. Hoffmann *et al.* (1992) evaluated the efficacy of transgenic tobacco plants contaning genes encoding *B. thuringiensis* δ-endotoxin or cowpea trypsin inhibitor against *H. zea* under field conditions and reported that the mortality of the larvae was high and the leaf damage was low for the genotypes contaning Bt gene as compared to control and CpTI genotype. Transgenic tobacco contaning *B. thuringiensis* (Bt) and cowpea trypsin inhibitor (CpTI) genes showed insecticidal activity towards *H. armigera*

(Zhao *et al.*, 1997). Mortality of the larvae was low on transgenic tobacco expressing Bt alone than the plants expressing both Bt and CpTI. It was concluded that gene pyramiding could be a valuable strategy for resistance management and the sustainable use of Bt transgenic crops. Santos *et al.* (1997) transformed *Landsberg erecta* plants with either Bt Cry1A (c) gene, or CpTI gene or for both genes and tested them against *S. exigua*, *H. zea*, *Pseudoplusia includens* and *H. virescens*. Both genes reduced growth of the species tested, but CrylA (c) was more effective in controlling caterpillar growth than the cowpea trypsin inhibitor gene. The resistance of plants with both transgenes was lower than that of plants expressing the CrylA (c) gene alone, but higher than that of plants expressing only the CpTI gene. Transgenic tobacco plants containing Bt or CpTI or both of the genes proved toxic to larvae of *H. armigera*. Plants with both transgenes had enhanced resistance compared to those with single transgenes (Zhao *et al.*, 1996). *Bacillus thuringiensis* Cry1A (c) protein expressed in transgenic cotton has biological activity specific for Lepidoptera and risks to beneficial non-Lepidoptera insect species are negligible (Sims, 1995).

Benedict *et al.* (1996) studied the field performance of transgenic cotton plants (BTK lines) for resistance to *H. virescens* and *H. zea* and reported that in BTK lines, the mean percent injury was 2.3 of the flower buds and 1.1 of the capsels, whereas in Coker 312 the mean percentage injury was 23 of the flower buds and 12 of the capsels. Average yield of Coker 312 was 1050 kg ha^{-1} and that of BTK was 1460 kg ha^{-1}. Tobacco and tomato plants expressing Cry 1A(b) and Cry 1A(c) genes have also been developed (Van der Salm *et al.*, 1994) to control lepidopteran insects. The expression of Cry 1A(b)-Cry 1A(c) genes provided protection against *S. exigua, M. sexta* and *H. virescens.*

Proteinase inhibitors

Plants use proteins as a part of their defense strategies. An interesting class of defense proteins are the inhibitors of digestive enzymes that occur in many plants. The two main classes of inhibitors discovered so far are the protease inhibitors and the amylase inhibitors. Among them, protease inhibitors play an important role in defense of plants against herbivorous insects. They act as competitive inhibitors of enzymes by binding tightly to the active site of the enzyme. The antimetabolic activity of the protease inhibitors is due to direct inhibition of larval proteolysis and utilization of proteins leading to the death of the larvae by slow starvation.

Proteinase inhibitors are widely distributed in the plant kingdom, particularly in seeds and tubers, where they often represent several percent of total protein (Liener and Kakade, 1969; Ryan, 1973; Richardson, 1977). They have been most extensively studied in the Leguminosae, Graminae and Solanaceae, presumably because of the large number of species in these families, which are important food crops (Richardson, 1980). According to specificity, proteinase inhibitors can be divided in four classes, inhibiting serine, cysteine, metallo- or aspartyl proteases. Several nonhomologous families of protease inhibitors are recognized among the animal, microorganism, and plant kingdoms (Laskowski *et al.*, 1980). In plants, about ten protease-inhibitor families have been recognized (Garcia *et al.*, 1987). These inhibitor families are specific for each of the four mechanistic classes of proteolytic enzymes, i.e. serine, cysteine, aspartic and metallo-proteases. Members of the serine and cysteine proteinase inhibitor families have been more relevant to the area of plant defense than metallo and aspartyl proteinase inhibitors, since only a few of these latter two families of inhibitors have been found in plants.

Serine proteinase inhibitors

More research has been carried out on members of the serine class of proteinases as compared to other proteinase inhibitors. Serine proteinases have been identified in extracts from the digestive tracts of insects from many families, particularly those of the Lepidoptera (Applebaum, 1985; Broadway, 1989; Houseman, 1989). In Lepidoptera, which includes a number of pests, the pH optima of the guts are in the alkaline range 9 to 11, where serine proteinase is most active. Additionally, serine proteinase inhibitors have anti-nutritional effects against several lepidopteran insect species (Applebaum, 1985; Broadway, 1986; Gatehouse, 1983; Hilder, 1987; Johnson, 1989; Shukle, 1983). There are about seven families of protein inhibitors present in the plants that inhibit serine proteinases. Out of these, soybean trypsin inhibitor (SBTI, Kunitz family), and Bowman- Birk proteinase inhibitor are very important.

Soybean trypsin inhibitor

Soybean trypsin inhibitor was the first plant inhibitor to be well characterized. Its isolation and crystallization from soybean and that of its complex with trypsin by M. Kunitz is one of the classic achievements of inhibitor chemistry (Kunitz, 1947). It has a molecular weight of 20,000 to 25,000 with relatively few disulphide bonds and posses a specificity, which is directed primarily towards trypsin. Trypsin (Mw 23,300) is the main intestinal digestive enzyme responsible for the hydrolysis of food proteins. It is a serine protease and hydrolyzes peptide bonds in which the carboxyl groups are contributed by the lysine and arginine residues. Due to the ability of this inhibitor to inhibit trypsin from the insect gut, it has received much attention as a target for control of insect pests.

Bowman-Birk proteinase inhibitor

These inhibitors are readily isolated from the seeds of all leguminous plants. Bowman first discovered them in soybean, and thereafter Birk purified and characterized them. They have a molecular weight of 6000 to 10,000 with a high proportion of cystine residues and are capable of inhibiting trypsin as well as chymotrypsin at independent sites.

Proteinase inhibitor in insect control

The idea of using proteinase inhibitors in insect control originated with the research of Mickel and Standish (1947), who found that larvae of certain pests do not develop normally when fed with soybean products. Lipke *et al.* (1954) found that a protein fraction from soybeans inhibited growth, as well as proteolytic activity *in vitro*, of the mealworm, *Tribolium confusum* Viggiani. Birk and Applebaum (1960) studied the effects of various proteinase inhibitors in soybean meal on both the development of *Tribolium castaneum* Herbst larvae and the proteolytic-enzyme activity in their midgut homogenates and found that only two fractions possessed inhibitory activity against the midgut enzyme of *T. castaneum* larvae. Birk *et al.* (1963) isolated a *Tribolium* proteinase inhibitor from other proteinase inhibitors and showed that the inhibitor could completely inhibit the midgut proteolytic activity of both *T. castaneum* and *T. confusum*. An inhibitor of *Tribolium* proteinase was also found in wheat (Applebaum and Konijn, 1966). It was inactive towards bovine trypsin and chymotrypsin.

The protective role for these proteins was proposed in 1972 when Ryan and co-workers discovered that damage to the leaves of certain solanaceous plants, either by insect feeding or mechanical wounding, induced the synthesis of protease inhibitors (Green *et al.*, 1972). Production of these inhibitors was shown to be a result of a wound hormone, proteinase inhibitor-

inducing factor (PIIF) that is released from the damaged leaves and translocated throughout the plants, where it initiates the synthesis and accumulation of inhibitors (Shumway *et al.*, 1976; Walker *et al.*, 1977; Brown *et al.*, 1985).

The protective role for protease inhibitors in the field resistance was demonstrated in 1979, when it was found that the levels of protease inhibitors (CpTI) present in seeds of a resistant line of cowpea, TVu 2027, was correlated with resistance to a major insect pest, *Callosobruchus maculatus* F. The resistant variety contained about twice the levels of inhibitors as compared to any other variety (Gatehouse *et al.*, 1979). A proteinase inhibitor active against tyrpsin and chymotrypsin was isolated by affinity chromatography. The antimetabolic effect of the inhibitor was confirmed in insect feeding trials with *C. maculatus* larvae. When the inhibitor was fed at 0.8% of the diet, none of the larvae survived, whereas at 0.1%, over 80% of the larvae survived and pupated. Various bioassays have been conducted using the proteinase inhibitors in artificial diets and their effects on the insect growth and development has been studied.

Steffens *et al.* (1978) reported that when soybean trypsin inhibitor (Kunitz) and a weak inhibitor of trypsin from corn were fed to larvae of European corn borer larvae at 2 to 5% of diets, SBTI inhibited growth of the larvae and delayed pupation, whereas the corn inhibitors had no effect on growth or metamorphosis of the larvae. Soybean trypsin inhibitor (Kunitz inhibitor) retarted larval growth of *M.sexta*, when added into an artificial diet at the 5% level (Shukle *et al.*, 1983). Broadway and Duffey (1986) tested the effect of purified SBTI and potato Inhibitor II (an inhibitor of both trypsin and chymotrypsin) on the growth and digestive physiology of larvae of *H. zea* and *S. exigua*. These proteins at levels of about 10% in the diet (1.2% caesin), inhibited growth of the larvae.

Soybean Kunitz trypsin inhibitor (SBTI) and Soybean Bowman-Birk trypsin-chymotrypsin inhibitor (SBBI) in artificial diet reduced total larval biomass and mean larval weights of *H. armigera*. These effects were much greater with dietary SBTI than with SBBI (Johnston *et al.*, 1993). Soybean trypsin inhibitor significantly affected the growth and digestive physiology of *H. armigera*. When incorporated into an artificial diet 0.84% (dry weight), SBTI significantly reduced the high alkaline trypsin-like enzyme activity by18% (Wang *et al.*, 1996). Soybean (Kunitz) trypsin inhibitor reduced the growth rate of the larvae of *S. litura* when incorporated into artificial diet at 0.2 % (w/v) and 0.5 % (w/v). The slowest growth rate and the lower weights were observed for larvae fed with 0.5 % SBTI concentrarion (McManus *et al.*, 1995). Soybean trypsin inhibitor (STI) in artificial diet at 0.84 to 4.2 % (dry weight) affected the growth and *in vivo* midgut proteinase activity of *H. armigera*. The retarding effect of STI on the growth of the larvae was significant, but not proportional to the dosage of STI (Wang *et al.*, 1995). Protease inhibitors in artificial diets at 0.33 and 0.66% affected the growth rate of codling moth larvae, *Cydia pomonella (*L.*)*. Potato proteinase inhibitor I was most effective in reducing growth rate, followed by soybean trypsin inhibitor (Markwick *et al.*, 1995).

The first successful example of genetic engineering of plants for insect resistance using genes of plant origin was achieved using a cowpea protease inhibitor (CpTI) gene (Hilder *et al.*, 1987). These workers transformed tobacco with a gene that encoded a cowpea trypsin inhibitor and reported that CpTI transformants, which expressed the foreign protein at nearly 1%, were relatively resistant to attack by the tobacco budworm, *H. virescens*. Over-expression of several inhibitors has been shown to afford protection in transgenic tobacco plants against attack by Lepidopteran larvae. Expression of CpTI in tobacco afforded significant protection in the field against *H. zea* (Hoffmann *et al.*, 1992). Johnson *et al.* (1989) transformed tobacco plants with genes encoding for the potato and tomato proteinase inhibitor II proteins (having chymotrypsin and trypsin inhibitor activities) and a tomato inhibitor I

protein having only chymotrypsin inhibitor activity. Leaves of plants expressing the inhibitor II proteins at levels of 50 μ g^{-1} tissue retarded the growth of larvae of *M. sexta* and at higher levels of 100 μ g^{-1} tissue, the larvae grew even less and some died. They concluded that decrease in the larval growth was roughly proportional to the level of PI-II being expressed. McManus *et al.* (1994) transformed tobacco with potato inhibitor II, which inhibits chymotrypsin. Larvae of *Chrysodeixis eriosma* (green lopper) grew slowly on leaf tissue from the transgenic plants than from non-transgenic plants, whereas no differences were observed in the growth rates of *S. litura* or *Thysanoplusia orichalcea* larvae fed on leaves from transgenic or non-transgenic plants.

Soybean trypsin inhibitor (STI) contaning transgenic tobacco plants showed high resistance to the larvae of *H. armigera*. (Gao *et al.*, 1998). Li *et al.* (1998) obtained transgenic cotton lines contaning Cowpea trypsin inhibitor (CpTI) gene and found them to be highly resistant to cotton bollworm. Transgenic rice plants contaning Soybean Kunitz trypsin inhibitor (SKTI) showed resistance to the brown planthopper *(Nilaparvata lugens Stal)* (Lee *et al.*1999). Transgenic poplar plants expressing a Kunitz proteinase inhibitor (Kti3) gene did not affect larval mortality, growth and pupal weights of *Lymantria dispar* (L). and *costera anastomosis* (Confalonieri *et al.*, 1998). Proteinase inhibitors from *Nicotiana alata* in artificial diet significantly reduced the growth of the native budworm larvae (*H. punctigera* Wallengern) and the black field cricket nymphs *(Teleogryllus commodus* Walker). When expressed in transgenic tobacco, these inhibitors showed significant differences in mortality and growth rate of *H. punctigera* larvae (Heath *et al.*, 1997).

Lectins

Many plant species contain carbohydrate-binding proteins, which are commonly referred to as either lectins or agglutinins. Lectins are proteins that

bind reversibly to specific mon- or oligosaccharides. The first description of a lectin dates back to 1888, when Stillmark published his dissertation about Ricin, a toxic ferment obtained from the seeds of *Ricinus communis* L. and some other Euphorbiaceae species (Stillmark, 1888). Stillmark's 'ricin' was a complex mixture of toxic ricin molecules and nontoxic agglutinins. His pioneering work was a milestone in biology because he was the first to link the toxicity of castor beans to the occurrence of a hemagglutinating proteinaceous factor. In 1898, Elfstrand introduced for the first time the term 'Blutkorperchenagglutinin' (hemagglutinin) as a common name for all plant proteins that cause clumping of cells (Elfstrand, 1898). The idea that toxicity is an intrinsic property of lectins was abandoned in the beginning of the century after Landsteiner and Raubitschek (1907) reported the presence of nontoxic lectins in the legumes *Phaseolus vulgaris* L. (bean), *Pisum sativum* L. (pea), *Lens culinaris* Medik (lentil), and *Vicia sativa* (Vetch). Following the work of Landsteiner and Raubitschek, many more nontoxic plant hemagglutinins were discovered. Eventually, it became evident that toxicity is the exception rather than the rule. The next milestone in the history of plant lectins was the finding that some hemagglutinins exhibit a clear preference towards erythrocytes of a particular human blood group within the ABO system (Renkonen, 1948; Boyd and Reguera, 1949). This discovery of blood group specificity led to the introduction of the novel term 'lectin' (from the Latin verb 'legere', which means 'to select'). Summer and Howell (1936) observed that cane sugar inhibited the agglutination activity of Concanavalin A (Con A), and later it was discovered that the agglutination properties of lectins are based on a specific sugar-binding activity (Watkins and Morgan, 1952).

According to the first proper definition, lectins are carbohydrate-binding proteins (or glycoproteins) of nonimmune origin that agglutinate cells and/or precipitate glycoconjugates (Goldstein *et al.*, 1980). Although this definition was approved by the Nomenclature Committee of the International Union of Biochemistry (Dixon, 1981), it had some shortcomings because it was

confined to multivalent carbohydrate-binding proteins. According to recent definition, plant lectins were defined as all plant proteins possessing atleast one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide (Peumans and Van Damme, 1995).

Lectins are found in many plant tissues and are abundant in the storage organs and protective structures of some plants. They have been isolated from various plant tissues, with seeds being the richest source (Etzler, 1986). This is especially true among plants in the Leguminosae family (Strosberg *et al.*, 1986).

Based on their overall structure lectins are subdivided as 'merolectins', 'hololectins', 'chimerolectins', and 'superlectins'. Merolectins are proteins that are built exclusively of a single carbohydrate-binding domain. They are monovalent and hence cannot precipitate glycoconjugates or agglutinate cells. Hevein, the small chitin-binding protein from the latex of the rubber tree (*Hevea brasiliensis*) (Van Parijs *et al.*, 1991) is a typical merolectin. Hololectins also are exclusively built up of carbohydrate-binding domains, but contain atleast two such domains that are either identical or very homologous and bind either the same or structurally similar sugars. This group comprises all lectins that have multiple binding sites and, hence, are capable of agglutinating cells or precipitating glycoconjugates. The majority of all known plant lectins are hololectins. Chimerolectins are fusion proteins possessing a carbohydrate-binding domain tandemly arrayed with an unrelated domain, which has a well-defined catalytic activity (or another biological activity) that acts independently of the carbohydrate-binding domain. Depending on the number of sugar-binding sites, chimerolectins behave as merolectins or hololectins. For instance, type 2 RJPs with two carbohydrate-binding sites on their B chain (e.g., ricin) agglutinates cells, whereas class I plant chitinases with a single chitin-binding domain do not. Like hololectins, superlectins consist exclusively of atleast two carbohydrate-binding domains. However, unlike the hololectins, the carbohydrate-binding domains of the superlectins

recognize structurally unrelated sugars. Therefore, superlectins can also be considered as a special group of chimerolectins composed of two tandemly arrayed, structurally and functionally different carbohydrate-binding domains.

Mode of action of plant lectins

The epithelial cells along the digestive tract of phytophagous insects are directly exposed to the diet and therefore are possible target sites for plant defence proteins. As glycoproteins are major constituents of these membranes, the luminal side of the gut is covered with potential binding sites of dietary lectins, resulting in repulsion of the insects, retarded growth or mortality. Although the mechanism of toxicity is based on the specific binding of the glycoconjugates in the gut of the insect, the exact mechanism of action of plant lectins is not known.

There are several possible interactions that could occur (Chrispeels and Raikhel, 1991; Czapla and Lang, 1990; Peumans and Van Damme, 1995a,b).

1. binding of the lectins to the chitin matrix of the peritrophic membrane,
2. binding of the lectin to glycoconjugates within the peritrophic membrane,
3. binding of the lectin to glycoconjugates exposed on the surface of the midgut epithelial cells,
4. binding of lectins to glycosylated digestive enzymes, and
5. binding of glycosylated proteins from the host plant preventing the protein's digestion.

Lectins as Insect control proteins

The effect of a lectin on the normal development of insects was first investigated in 1976, when Janzen *et al.* reported that phytohemagglutinin (PHA) from black bean, *Phaseolus vulgaris* L., produced deleterious effects on the larvae of bruchid beetle *Callosobruchus maculates* F. (Cowpea weevil) (Janzen *et al.*, 1976). Female weevils oviposited on the artificial seeds containing phytohemagglutinin (PHA) at concentrations 0.1, 1 and 5%. Control artificial seeds produced an average of 4.5 beetles per seed. Seeds with 0.1% phytohemagglutinin produce 3.6 beetles per seed, while seeds with 1% phytohemagglutinin produced two small adults, and no adult beetles emerged on diets containing 5% PHA. Gatehouse *et al.* (1984) reported that lectin from seeds of *Phaseolus vulgaris* L. affected the growth of larvae of the bruchid beetle.

Murdock *et al.* (1990) screened seventeen plant lectins to determine their biological activity against *C. maculatus* F. and showed that lectins from osage orange (*Maclura pomifera* Rafinesque), peanut (*Arachis hypogaea* L.), potato (*Solanum tuberosum* L.), jimson weed (*Datura stramonium* L.), and wheat germ (*Triticum aestivum* L.) delayed the developmental time of *C. maculatus* at dietary levels of 0.2 and 1.0% (w/w).

Huesing *et al.* (1991a) isolated three wheat germ isolectins and showed that each of these isolectins were equally effective against the cowpea weevil. N-acetylglucosamine (G/c NAC) binding lectins from *Oryza sativa L.* (rice) and *Urtica dioica* (stinging nettle) also showed increased mortality and increased development time when fed to the cowpea weevil (Huesing *et al.*, 1991b). Activity of rice lectin was similar to WGA, while the stinging nettle was approximately 2 to 4 times less effective than WGA.

Gatehouse *et al.* (1992) showed that lectins from *Allium sativum* (garlic) and *Galanthus nivalis* (snowdrop) affected the survival of the cowpea

weevil larvae. Larvae reared on artificial seeds containing 2% lectin suffered 90% mortality compared to the control larvae. Larval mortality was greater than 50% at 1% level.

Czapla and Lang (1990) tested twenty-six plant lectins for anti-insect properties against neonate European corn borer (*Ostrinia nubilalis* Hubner) larvae and reported that only three lectins were effective at a 2% topical level. They were WGA, *Bauhinia purpurea* L. *agglutinin* (BPA) and the lectin from *Ricinus communis* L. Lectins from castor bean, pokeweed (*Phytolaca americana* L.), and the green marine algae *Codium fragile* (Surinagar) were toxic to the neonate southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, when applied topically (2%) to the artificial diet (Czapla and Lang, 1990).

Habibi *et al.* (1993) studied fourteen plant lectins for their activity against potato leafhopper (*Empoasca fabae* Harris.) and showed that six lectins (wheat germ, jackfruit, pea, lentil, red kidney bean, and horse gram) reduced the survival of potato leafhoppers, when incorporated into the artificial diet at a concentration of 0.2 to 1.5% (w/v).

Powell *et al.* (1993) determined that GNA and WGA showed antimetabolic effects against the first- and third-instar nymphs of rice brown planthopper (*Nilaparvata lugens stal*) when incorporated into artificial diet at 0.1% (w/v). GNA also showed inhibitory effect towards third-instar nymphs of the rice green leafhopper (*Nephotettix cinciteps* Ulher). GNA and WGA reduced honeydew excretion levels of adult rice brown planthopper, when added to artificial diet at 0.1% (w/v) (Powell *et al.*, 1995).

Powell *et al.* (1995) tested three mannose-binding lectins and two N-acetyl glucosamine binding lectins towards the third-instar nymphs of the rice brown plant hopper and showed that among the mannose-specific lectin, GNA was the most toxic. *Narcissus pseudonarcissus agglutinin* (NPA) and *Allium sativum agglutinin* (ASA) showed significant antimetabolic effect towards

brown planthopper, but were less effective than GNA. The two N-acetyl glucosamine binding lectins, the dimeric *Oryza sativa agglutinin* (OSA), and the monomeric *Urtica dioica agglutinin* (UDA) showed no antimetabolic effect towards brown planthopper when tested at concentration of 0.1% w/v.

Shukle and Murdock (1983) conducted bioassays with the soybean lectin and reported that it inhibited larval growth of *M. sexta* L., when incorporated into an artificial diet at a 1% level. GNA reduced weight gain and survival when bioassayed against *Spodoptera littoralis* Boisduval at the 5% level (Gatehouse *et al.*, 1992).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The antifeedant and the food consumption and utilization studies were conducted using putative transgenic tobacco plants. The tobacco plants were transformed with pHs 723:Bt carrying Bt Cry 1A(b) and pHs 737:SBTI genes through *Agrobacterium tumefacience* mediated genetic transformation. The independent transformants of Bt and SBTI tested are listed in Table 1.

The antibiotic studies were conducted using lectins and soybean trypsin inhibitor obtained from Sigma Chemical Co. (St Louis, Mo. U.S.A). These studies were carried out in the laboratory at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India.

Antifeedant Studies

The antifeedant studies were conducted using no-choice and dual-choice assays at 27±1°C under laboratory conditions.

Transformed and the nontransformed plants were raised on medium size pots (25 cm diameter) in the greenhouse at atmospheric conditions (33±5°C, and 65 ± 5 % RH). Potting mixture consisted of 2:1 ratio of red soil: FYM (farm yard manure). Plants were thinned after one week of crop emergence. Two week old plants were transplanted individually into small pots (14 cm diameter) containing a mixture of red soil and FYM (2:1). Urea @10 g per pot was applied one week after transplanting. The plants were watered daily as needed.

The *H. armigera* larvae used in the experiments were reared under laboratory conditions (27 ± 1°C and 60 ± 5 % RH) on artificial diet (Table 2).

Table 1. Independent transformants of Bt and SBTI used in the study

Transformants	Gene involved
723:Bt 1	Cry 1A(b)
723:Bt 3	Cry 1A(b)
723:Bt 4	Cry 1A(b)
723:Bt 7	Cry 1A(b)
723:Bt 8	Cry 1A(b)
723:Bt 10	Cry 1A(b)
723:Bt 12	Cry 1A(b)
737:SBTI 1	SBTI
737:SBTI 5	SBTI
737:SBTI 11	SBTI
737:SBIT 12	SBTI

Table 2. Ingredients of artificial diet used for rearing *H.armigera* in the laboratory (ICRISAT, Patancheru, India).

Ingredients	Amount
Chickpea flour	300.00 g
Ascorbic acid	4.70 g
Methyl-p-hydroxygenzoate	5.00 g
Sorbic acid	3.00 g
Auromycin powder	11.50 g
Vitamin stock solution	10.00 ml
Water	450.00 ml
Yeast	48.00 g
Agar	17.30 g
Water (for yeast/agar)	800.00 ml

No-choice Assay

The no-choice assay was conducted using the first-instar and the third-instar larvae of *H. armigera*.

No-choice assay with first-instar larvae

Fully expanded, mature leaves were used in this experiment. Leaf discs were cut with a no. 4 cork borer and weighed on a Mettler balance ®. The discs were placed in plastic cups of 3.5 cm diameter and 4 cm height. Filter paper (3 cm diameter) was placed on the bottom and top of the cup .The one on the top was soaked with 1 ml water to keep the larvae water satiated. Ten newly hatched first-instar larvae were released on the leaf discs, using a fine camel hairbrush. Ten discs were kept in each treatment in a similar manner, but without larvae to determine the natural loss in leaf mass.

Observations. Larval mortality was recorded at 24, 48 and 72 h. The leaf discs were rated visually for insect feeding on a 1 to 9 scale (1 = < 10 % leaf disc area consumed, 2 = 11 - 20, 3 = 21 - 30, 4 = 31 - 40, 5 = 41 - 50, 6 = 51 - 60, 7 = 61 - 70, 8 = 71 - 80, and 9 = >80 % leaf disc area consumed). Leaf discs and the larval weights were recorded after 72 h of infestation.

No-choice assay with third-instar larvae

Leaf discs were cut with a no. 4 cork borer and placed in the Petri dishes (10 cm diameter and 2 cm depth). Filter paper (9 cm diameter) was placed on the bottom and the top of Petri dishes. The one in the top was soaked with 2 ml water to keep the larvae water satiated. The third-instar larvae were weighed after starving for 4 h and confined individually with the leaf discs for 2 days.

Observations. The leaf discs were rated visually on a 1 to 9 scale after 24 and 48 h of infestation as described above. After 48 h, the larvae were removed from the Petri dishes and weighed. Each leaf discs was passed through a leaf area meter to determine the unconsumed leaf disc area.

Dual-choice assay

For dual choice tests, the leaf discs were cut with a no. 4 cork borer and centered in an apposed arrangement, 5 mm apart, in a 10 cm diameter Petri dish. Filter paper (9 cm diameter) was placed on the bottom and the top of Petri dishes. The one in the top was soaked with 2 ml water to keep the larvae water satiated. Discs were positioned on the filter paper using a shortened no.2 insect pin. The third-instar larvae were weighed after starving for 4 h and confined individually with a leaf discs for 2 days. There were 5 replications for each treatment.

Observations. The leaf discs were rated visually for insect feeding on 1 to 9 scale after 24 and 48 h of infestation. After 48 h, the larvae were removed from the Petri dishes, starved for 4 h and weighed. Each discs was passed through a leaf area meter to determine the unconsumed leaf disc area

Effect of soybean trypsin inhibitor and plant lectins on survival and development of *H. armigera*

The lectins and the soybean trypsin inhibitor used in the experiment were purchased from Sigma Chemical Co. (St Louis, Mo. U.S.A). Names, molecular weights, and carbohydrate-specificity of the lectins are listed in Table 3. The chemicals were tested at 0.1% concentration. Fifty mg of each lectin was dissolved in five ml of phosphate saline buffer (pH 6.8). The stock solution for the buffer was prepared in the following way.

A .2 M solution of monobasic sodium phosphate (27.8 g in 1000 ml).

B .2 M solution of diabasic sodium phosphate (53.65 g of Na2HPO4 $7H_2O$ or 71.7 g of Na_2HPO_4 $12H_2O$ in 1000 ml).

Phosphate saline buffer for dissolving the lectins was prepared by mixing 51.0 ml of A + 49.0 ml of B, diluted to a total of 200 ml. Similarly fifty mg of soybean trypsin inhibitor was dissolved in five ml of distilled water. Five ml of the dissolved lectins and the soybean trypsin inhibitor was added to forty-five ml of artificial diet of *H. armigera,* and stirred on the magnetic stirrer. The

Table 3. Lectins tested against *H. armigera*

Common name	Taxonomic name	Molecular weights X 10^{-3}	Sugar specificity
Concanavalin A	*Canavalia ensiformis*	102	α-mannose α-glucose
Chickpea	*Cicer arietinum*	44	Fetuin
Lentil	*Lens culinaris*	49	α-mannose
Jacalin	*Artocarpus integrifolia*	42	α-galactose
Soybean	*Glycine max*	110	N-acetylgalactosamine (GalNAc)
Peanut	*Arachis hypogaea*	120	β-galactose (1 → 3) N-acetylgalactosamine (galNAc)
Snowdrop	*Galanthus nivalis*	52	Non-reduc. α-mannose

control treatment consisted of five ml of distilled water or five ml buffer of 6.8 pH added to forty-five ml of diet. Diet thus prepared was dispensed in aliquots of five ml into glass vials (2.5 cm diameter and 8 cm length) and allowed to cool for 3 h on the lab table. Five first-instar larvae were released in each vial. There were ten replications for each treatment.

Observations. Larval weights and mortality were recorded on fifth day. One larva in each vial was placed back into the diet, and later observations were recorded on date of pupation and adult emergence. Pupal weight was recorded one day after pupation. The remaining larvae were killed in chloroform after 4 h of starvation, and then dried in an oven at 65°C for 72h. Oven dry weights of the larvae were recorded.

Studies on Food Consumption and Utilisation

First-instar larvae of *H. armigera* were reared on nontransformed and transformed plants. Newly moulted third-instar larvae were used for studying the consumption and utilization of food. Larvae reared on nontransformed plants were confined to the leaves of nontransformed and transformed plants, whereas larvae reared on transformed plants were confined to the leaves of the respective transformed plant and the nontransformed plants. Freshly detached leaves were weighed on a Mettler balance, and placed in the Petri dishes (10 cm diameter and 2 cm depth). Filter paper (9 cm diameter) was placed on the bottom and the top of Petri plates. The one on the top was soaked with 2 ml water to keep the larvae water satiated. The third-instar larvae were weighed after starving for 4 h and confined individually with the leaf for three days. Five leaves of each plant were kept in a similar manner, but without larvae, to determine the natural loss in leaf mass. The Petri plates were kept in a plastic tray (35 x 25 x 5) at 27±1°C under laboratory conditions.

Observations. Three days after confinement, the larvae, uneaten food, and the control leaves were weighed, and then placed in an oven at 65°C for 72 h for drying. The larvae were killed with chloroform before drying. Dry mass of five larvae reared on nontransformed and transformed plants were also determined individually at the beginning of the experiment to compute a mean dry mass of larva before feeding. Natural loss of leaf mass was also recorded and the actual mass of the uneaten food was corrected in relation to natural loss of leaf mass.

Various indices of food consumption and utilization used are:

$$\text{Consumption index} = \frac{\text{Food consumed}}{\text{Duration of feeding period x Mean larval weight}}$$

$$\text{Growth rate} = \frac{\text{Weight gain by larvae during the feeding period}}{\text{Duration of the feeding period x Mean larval weight}} \times 100$$

$$\text{Efficiency of conversion of ingested food} = \frac{\text{Weight gain by larvae during the feeding period}}{\text{Weight of food consumed}} \times 100$$

$$\text{Efficiency of conversion of digested food} = \frac{\text{Weight gain by larvae during the feeding period}}{\text{Weight of food consumed - Weight of faeces}} \times 100$$

$$\text{Approximate digestibility} = \frac{\text{Weight of food consumed - Weight of faeces}}{\text{Weight of food consumed}} \times 100$$

Effect of SBTI and Bt transformed plants on the biology of *H.armigera*

Leaves of the nontransformed and transformed plants were collected from plants raised in the greenhouse. Leaves were cut into pieces and placed in plastic cups (3.5 cm diameter). Twenty-five first-instar larvae were reared individually in the cups for each treatment. Food was changed every alternate day.

Observations. Observations were recorded on larval survival. Duration of larval period was recorded in terms of number of days from the release of the larvae till pupation. Number of adults emerged from the pupae were also recorded in each treatment.

Statistical analysis

The data were subjected to analysis of variance. Significance of differences between the treatment means was tested by F - test, while the treatment means were compared by least significant difference (LSD) at P 0.05. In dual choice tests, the treatment means were compared by student's t - test.

CHAPTER IV

RESULTS

RESULTS

Antifeedant activity towards first-instar larvae of *H. armigera* (no-choice tests)

Studies on antifeedant activity of the transformed plants towards first-instar larvae of *H. armigera* indicated that 723 Bt 1, 723 Bt 4, 723 Bt 3, 723 Bt 7, 737 SBTI 5 and 737 SBTI 1 reduced the leaf feed significantly over the nontransformed control (Table 4). Larval mortality was higher in larvae fed on 723 Bt 1. 723 Bt 4, 723 Bt 7, 723 Bt 10, and 737 SBTI 5 than on the control plants. Lines 723 Bt 3, 723 Bt 8, 723 Bt 12, 737 SBTI 1, 737 SBTI 11 and 737 SBTI 12 showed moderate levels of larval mortality (46.0 to 59.2%).

Antifeedant activity towards third-instar larvae of *H.armigera* (no-choice tests)

Visual damage rating was lower on Bt and SBTI transformed plants 723 Bt 4, 723 Bt 7, 723 Bt 8, 723 Bt 1, and 737 SBTI 5 compared to the nontransformed control plants at 24 and 48 h after initiating the experiment (Table 5). Highest antifeedant activity was observed in 723 Bt 4. There was a significant reduction in leaf disc consumption by the third-instar larvae on 723 Bt 4, 723 Bt 1, 723 Bt 7, 723 Bt 8, 723 Bt 10 and 737 SBTI 5 as compared to the non-transformed control (2.07 cm^2 unconsumed leaf disc area). Larval weight was significantly lower in the larvae fed on 723 Bt 4, 723 Bt 7, 723 Bt 8, 723 Bt 1, and 737 SBTI 5 (1.4 to 2.7 mg per larvae) as compared to those reared on the non-transformed plants (9.3 mg per larvae).

Antifeedant activity of tobacco lines transformed with Cry 1A(b) Bt and SBTI genes towards third-instar larvae of *H. armigera* in dual-choice assay.

In dual-choice tests, the feeding by the third-instar larvae of *H. armigera* was significantly greater on the control discs than those of the transformed plants of 723 Bt 1, 737 SBTI 12, 723 Bt 12, 737 SBTI 5, 723 Bt 10, 737 SBTI 11, 723 Bt 8, at 24 and 48 h after initiating the experiment (Table 6). Although the

Table 4. Antifeedant activity of eleven tobacco lines transformed with Cry 1A(b) Bt and SBTI genes towards the first-instar larvae of *H. armigera* under no choice conditions (ICRISAT, Patancheru, 2000).

Tobacco line	Damage rating (DR)	Larval weight (mg)	Larval mortality %			Consumed leaf disc weight (mg)	Natural loss in leaf weight (mg)	Weight of food consumed (mg)
			24 h	48 h	72 h			
723 Bt 1	1.00	1.00	27.00	51.20	72.80	3.70	0.80	2.90
723 Bt 3	1.60	1.40	9.00	26.00	59.20	5.40	0.90	4.50
723 Bt 4	1.60	0.70	23.00	46.00	68.00	4.90	0.90	4.00
723 Bt 7	1.80	0.60	22.00	41.50	67.20	6.80	1.00	5.90
723 Bt 8	2.50	1.00	17.00	31.00	61.80	10.40	0.90	9.50
723 Bt 10	2.10	1.00	25 00	40.00	65 30	9.00	0.90	8.10
723 Bt 12	3.30	1.20	19.00	40.00	49.00	10.60	1.00	9.50
737 SBTI 1	1.50	1.10	16.00	37.50	53.50	4.20	0.90	3.30
737 SBTI 5	1.40	1.20	28.00	53.00	61.00	3.80	1.00	2.80
737 SBTI 11	2.60	1.20	8.20	32.60	56.40	8.80	0.80	8.10
737 SBTI 12	3.80	1.60	11.00	37.00	46.00	16.20	0.90	15.30
Control	3.30	3.20	3.00	11.00	21.00	16.30	1.10	15.30
SE ±	0.49	0.20	4.05	4.90	4.70	3.00	0.07	3.00
LSD (5%)	1.35	0.55	11.19	13.54	12.99	8.29	0.19	8.29
CV%	70.10	52.70	73.90	41.60	26.20	102.90	24.50	115.50

DR (1 = <10% leaf disc area consumed, and 9 =>80% leaf disc area consumed).

Table 5. Antifeedant activity of eleven tobacco lines transformed with Cry 1A(b) Bt and SBTI genes towards the third-instar larvae of *H. armigera* under no-choice conditions (ICRISAT, Patancheru, 2000).

Tobacco line	Damage rating (DR)		Unconsumed leaf disc area (cm^2)	Weight gain after 48 h (mg)
	24 h	48 h		
723 Bt 1	1.70	2.10	3.52	2.50
723 Bt 3	3.75	4.00	2.87	8.30
723 Bt 4	1.15	1.75	3.60	1.40
723 Bt 7	1.60	2.25	3.51	1.80
723 Bt 8	1.85	3.20	3.40	2.40
723 Bt 10	1.75	3.75	3.12	4.90
723 Bt 12	3.10	4.50	2.74	6.70
737 SBTI 1	2.90	4.35	2.85	6.10
737 SBTI 5	1.75	2.55	3.38	2.70
737 SBTI 11	3.72	5.89	2.22	10.70
737 SBTI 12	3.25	4.55	2.77	6.00
Control	4.35	6.20	2.08	9.30
SE ±	0.48	0.53	0.29	1.00
LSD (5%)	1.34	1.49	0.61	3.00
CV%	58.70	44.80	23.00	63.40

DR (1 = <10% leaf disc area consumed, and 9 =>80% leaf disc area consumed).

Table 6. Antifeedant activity of eleven tobacco lines transformed with Cry 1A (b) and SBTI genes towards the third-instar larvae of *H. armigera* in dual-choice tests (ICRISAT, Patancheru, 2000).

Comparison	Damage rating (24 h)		t value	Damage rating (48 h)		t value	Unconsumed leaf disc area (48 h)		t value
	Control	Treatment		Control	Treatment		Control	Treatment	
723 Bt 1 vs Control	2.80	0.20	4.22*	5.8	0.4	4.56*	1.874	3.93	-3.45*
723 Bt 3 vs Control	4.60	0.00	*	5.4	3.4	0.78	1.832	2.59	-0.65
723 Bt 4 vs Control	1.20	1.60	-0.34	5.6	2	1.67	1.942	3.326	-1.56
723 Bt 7 vs Control	0.80	1.40	-0.85	4	3.4	0.28	2.578	3.136	-0.67
723 Bt 8 vs Control	3.80	0.60	3.37*	7.8	1.6	4.38*	0.69	3.728	-4.93*
723 Bt 10 vs Control	3.60	0.40	5.66*	8.6	1.2	8.84*	0.392	3.796	-10.2*
723 Bt 12 vs Control	1.20	0.00	*	8.4	1.2	6.86*	0.554	3.554	-5.81*
737 SBTI 1 vs Control	2.20	0.40	1.90	7	3.5	2.05	1.427	2.913	-1.75
737 SBTI 5 vs Control	3.40	0.20	3.58*	9	1	14.61*	0.334	3.686	-13.08*
737 SBTI 11 vs Control	3.00	0.40	3.2*	6	1.6	2.65*	1.68	3.53	-2.24
737 SBTI 12 vs Control	1.80	0.20	2.31	2.2	0.4	2.32	3.35	3.87	-2.3

* = t value significant at P 0.05

differences in leaf feeding were not statistically significant, 723 Bt 3, 723 Bt 4, 737 SBTI 1 also showed some antifeedant activity towards the third-instar larvae.

Effect of different soybean trypsin inhibitor and plant lectins on survival and development of *H. armigera*

Percentage of larval survival varied from 49% in larvae reared on diet impregnated with SBTI to 90% in larvae reared on the control diet. Amongst the lectins tested, larval survival was significantly lower in larvae reared on diet impregnated with SBTI (49%), snowdrop (64%), chickpea (65%) and jacalin (78%) (Table 7). Concanavalin A, lentil, soybean, and peanut lectins did not affect the survival of *H. armigera* larvae. Fresh and dry mass of the larvae was lower on control diet compared to the larvae reared on diets containing SBTI and plant lectins. Lectins did not affect the weight gain of the larvae. Pupal mass varied from 272.6 mg in larvae reared on diet having chickpea lectin to 359.2 mg in larvae reared on diet having snowdrop lectin. Low pupal mass was recorded in larvae reared on diet having chickpea lectin (272.6 mg) as compared to those reared on the control diet (335.4 mg). In snowdrop and peanut lectins, the pupal mass was more as compared to the larvae reared on the control diet.

Percentage pupation

Percentage pupation (of the total larvae released) was lowest in larvae reared on diet impregnated with SBTI (40%). Among the lectins, low pupation was observed in larvae reared on diet impregnated with chickpea (50%), snowdrop (60%) and soybean lectins (70%) (Figure 1).

Adult emergence

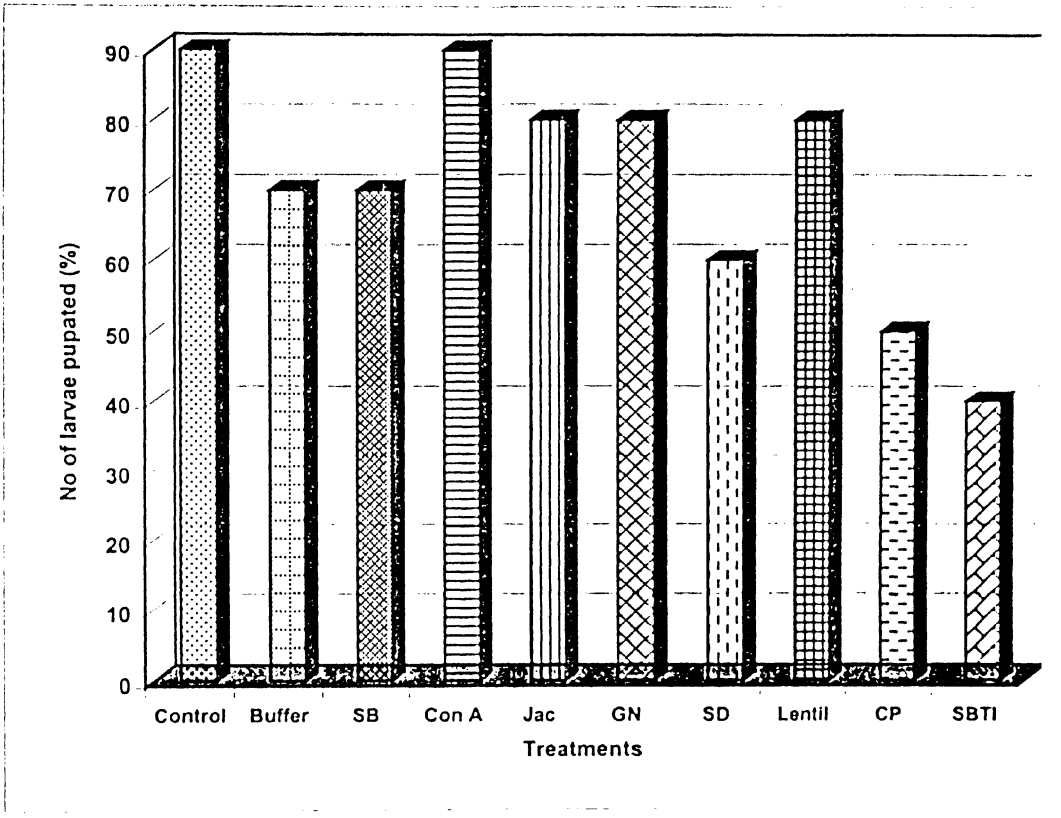
Percentage adult emergence (of the total larvae released) was lowest in larvae reared on diet having snowdrop lectin (20%), followed by those reared on SBTI (30%) and chickpea lectin (30%) (Figure 2). Low adult emergence

Table 7. Effect of plant lectins and soybean trypsin inhibitor on survival and development of *H. armigera* (ICRISAT, Patancheru, 2000)

Treatment	Dosage (mg/50 ml of diet)	Larvae survived on 5th day* (%)	Fresh mass of larvae (mg)	Dry mass of larvae (mg)	Pupal mass (mg)
Concanavalin A	50 mg	90 (76.72)	4.20	0.50	322.2
Lentil lectin	50 mg	90 (76.72)	4.00	0.60	323.6
Jacalin lectin	50 mg	78 (69.23)	4.50	0.60	325.1
Soybean lectin	50 mg	86 (74.18)	6.70	0.70	341.8
Peanut lectin	50 mg	84 (73.03)	4.60	0.60	349.5
Snowdrop lectin	50 mg	64 (55.03)	5.40	0.70	359.2
Chickpea lectin	50 mg	65 (61.98)	7.30	0.80	272.6
SBTI	50 mg	49 (44.33)	3.50	0.50	323.5
Buffer	-	86 (75.69)	1.20	0.20	336.7
Control	-	90 (81.00)	2.40	0.50	335.4
SE±	-	6.7 (5.76)	0.80	0.07	8.00
LSD (5%)	-	18.52 (15.92)	2.21	0.19	22.11
CV%	-	27.1 (26.5)	58.20	41.20	7.80

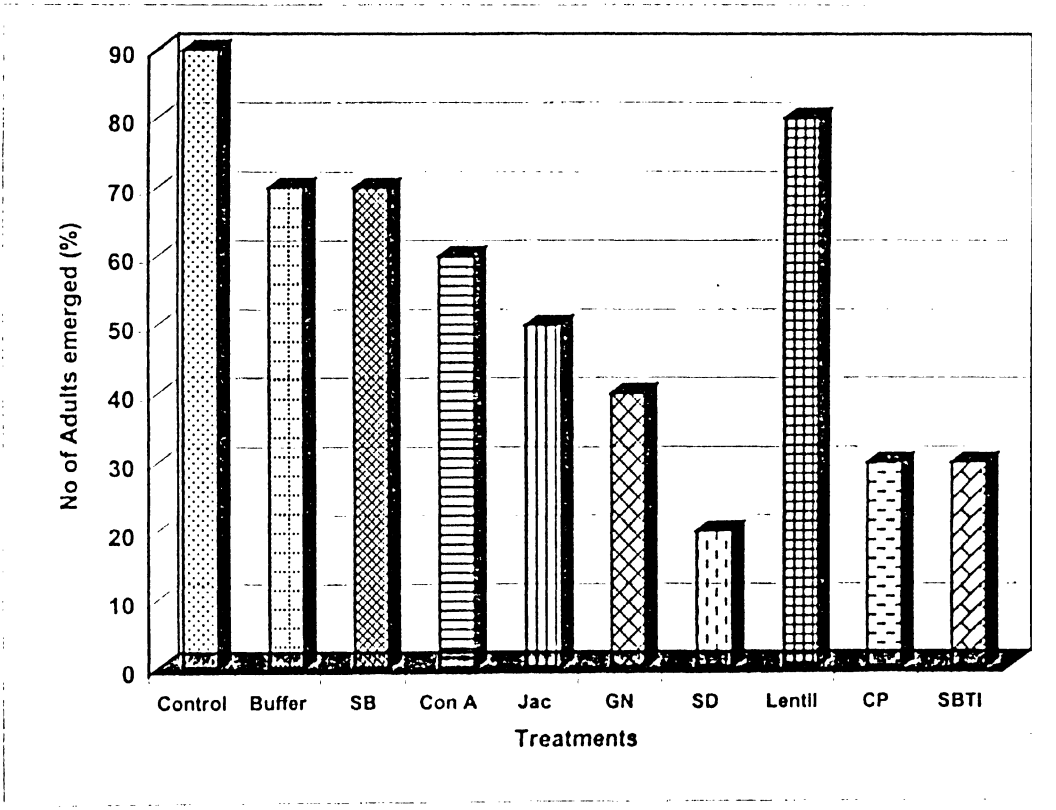
* Values in parantheses are Angular transformed values.

Fig.1. Effect of plant lectins and SBTI on pupation of *H. armigera* (50 mg/50 ml of diet) (ICRISAT, Patancheru, 2000)



SB = Soybean, Con A = Concanavalin A, Jac = Jacalin, GN = Groundnut, SD = Snowdrop, and CP = Chickpea lectin, and SBTI = Soybean trypsin inhibitor

Figure. 2. Effect of different plant lectins and SBTI on adult emergence of *H. armigera* (50 mg/50 ml diet) (ICRISAT, Patancheru, 2000).



SB = Soybean, Con A = Concanavalin A, Jac = Jacalin, GN = Groundnut,
SD = Snowdrop, and CP = Chickpea lectin, and SBTI = Soybean trypsin inhibitor

was also observed in diets having groundnut, jacalin, concanavalin A, soybean, and lentil lectins as compared to the untreated control (90%).

Consumption, digestion and utilization of food by the third-instar larvae of *H. armigera* on Bt and SBTI Transgenic Plants (wet weight basis)

No significant differences were observed in mass of food consumed by the third-instar larvae of *H. armigera* when reared on transformed plants compared to the control plants (Table 8). Larvae consumed more food per unit of bodymass (CI) and gained more weight when reared on the control plants compared to those reared on transformed plants. Except 737 SBTI 11, growth rate (GR) was significantly more in the larvae reared on control plants compared to those reared on the transformed plants (Table 9). Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) into .body matter was significantly greater in larvae fed on transformed plants in comparison to those fed on the control plants. Approximate digestibility was significantly low in the larvae fed on transformed plants as compared to those fed on control plants.

Food consumption in larvae reared on different transgenic lines (wet weight basis)

Mass of food consumed by the third-instar larvae of *H. armigera* was significantly greater in larvae reared on 737 SBTI 11 as compared to those fed on control plants, whereas in case of larvae reared on 723 Bt 12 and 723 Bt 7, the mass of food consumed was significantly lower on the transgenic plants as compared to the control plants. No significant differences were observed in mass of food consumed in case of other transgenic plants as compared to the control plants (Table 10). Weight gained by larvae was significantly lower in larvae reared on 723 Bt 12, 723 Bt 4 and 723 Bt 7 as compared to the controls, whereas in case of larvae reared on 737 SBTI 12 and 737 SBTI 11, the mass of larvae was more as compared to that on the control plants. On 737 SBTI 11, larvae consumed more food per unit of body

mass (CI) compared to the control plants. On 723 Bt 12 and 723 Bt 7, CI was significantly lower as compared to the control plants (Table 11). Differences in growth rate (GR) between transformed and the control plants were significant in all transgenic lines, except 737 SBTI 12. Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) was significantly higher in case of larvae reared on 723 Bt 12. On 737 SBTI 11 the ECI and ECD values were significantly lower in larvae reared on these lines as compared to those fed on the control plants. Approximate digestibility (AD) was significantly lower in case of larvae fed on 723 Bt 12 plants as compared to those fed on the control plants.

Consumption, digestion and utilization of food by the third-instar larvae of *H.armigera* on Bt and SBTI transgenic plants (dry weight basis)

Significant differences were observed in mass of food consumed by the third-instar larvae of *H. armigera* when reared on transformed plants compared to the nontransformed control plants (Table 12). Weight gained was significantly lower in larvae reared on transgenic plants as compared to the control plants. Larvae consumed more food per unit of bodymass (CI) and gained more weight when reared on the control plants compared to those reared on transformed plants. Growth rate (GR) was significantly more in the larvae reared on control plants compared to those reared on the transformed plants (Table 13). Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) into body matter was significantly greater in larvae fed on transformed plants in comparison to those fed on the control plants. Approximate digestibility was significantly lower in the larvae fed on transformed plants as compared to those fed on control plants.

Food consumption in larvae reared on different transgenic lines (dry weight basis)

Mass of food consumed by the third-instar larvae of *H. armigera* was significantly greater in larvae reared on 737 SBTI 11 and 737 SBTI 12 as

compared to those fed on control plants, whereas in case of larvae reared on 723 Bt 12 and 723 Bt 7, the mass of food consumed was significantly lower on the transgenic plants as compared to the control plants. No significant differences were observed in mass of food consumed in case of other transgenic plants as compared to the control plants (Table 14). Weight gained by larvae was significantly lower in larvae reared on 727 Bt 12, 723 Bt 4 and 723 Bt 7 as compared to the controls, whereas in case of larvae reared on 737 SBTI 12, the mass of larvae was greater as compared to the control plants. On 737 SBTI 11 and 723 Bt 7 larvae consumed more food per unit of body mass (CI) compared to the control plants. On 723 Bt 12, the CI was significantly lower as compared to the control plants (Table 15). Growth rate was significantly greater in control plants compared to the transformed plants in lines 723 Bt 4, 723 Bt 12 and 737 SBTI 11, whereas in 737 SBTI 12 the growth rate was more on transformed plants as compared to the control plants. Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) was significantly higher in case of larvae reared on 723 Bt 12. On 737 SBTI 11 and 723 Bt 7, the ECI and ECD values were significantly lower in larvae reared on these lines as compared to those fed on the control plants. Approximate digestibility (AD) was significantly lower in case of larvae fed on 723 Bt 1 and 723 Bt 12 plants as compared to those fed on the control plants, whereas on 723 Bt 7 AD was significantly more as compared to the control.

Effect of SBTI and Bt transformed plants on the biology of *H. armigera*

Larval mortality

Percentage larval mortality varied from 20% on untransformed control plants to 84% on the transformed plants. Larval mortality was greater in 737 SBTI 11, 723 Bt 4 and 737 SBTI 12 transformed plants. Larval mortality was more than 50% on 723 Bt 1, 723 Bt 7, and 737 SBTI 1 transformed lines. In all the transformants, larval survival declined from 3 to 15 days after infestation (Figure 3).

Table 8. Food consumption, weight gain, and amount of faeces produced by the third-instar larvae of *H. armigera* reared on *Cry 1A(b)* and *SBTI* transgenic tobacco plants (wet weight basis) (ICRISAT, Patancheru, 2000).

Treatment	Mass of food offered (g)	Mass of unconsumed food (g)	Mass of consumed food (g)	Natural loss of weight (g)	Corrected mass of food consumed (g)	Initial mass of larvae (g)	Mass of larvae after the feeding period (g)	Larval mass gain (g)	Mass of faeces produced (g)
B: 1	2.6210	2.5265	0.0944	0.00008	0.0944	0.0025	0.0282	0.0256	0.0400
B: 4	3.1368	3.0754	0.0614	0.00004	0.0614	0.0021	0.0219	0.0198	0.0160
B: 7	2.5911	2.5203	0.0708	0.00005	0.0708	0.0035	0.0266	0.0231	0.0214
B: 12	3.5590	3.4316	0.1274	0.00009	0.1274	0.0037	0.0382	0.0345	0.0311
SBTI 11	2.5574	2.4973	0.0601	0.00004	0.0601	0.0023	0.0184	0.0186	0.0179
SBTI 12	2.8521	2.7524	0.0997	0.00007	0.0997	0.0034	0.0241	0.0207	0.0192
Control	2.1378	1.8396	0.2982	0.00024	0.2982	0.0021	0.0558	0.0537	0.0574
SE±	0.3100	0.3000	0.2900	0.0000	0.0300	0.0003	0.0070	0.0070	0.0090
LSD (5%)	0.9004	0.8714	0.8423	0.0000	0.0871	0.0009	0.0203	0.0203	0.0261
CV%	25.2	25.5	54.7	~~56.4~~ 0.000	54.7	26.6	50.1	53.2	69.1

Table 9. Consumption, digestion, and utilization of food by the third-instar larvae of *H. armigera* fed on Cry 1A(b) and SBTI transgenic tobacco plants (wet weight basis) (ICRISAT, Patancheru, 2000).

Treatment	Consumption index (CI)	Growth rate (GR%)	Efficiency of conversion of ingested food (ECI%)	Efficiency of conversion of digested food (ECD%)	Approximate digestibility (AD%)
Bt 1	1.9628	48.4822	25.6815	42.5885	61.7141
Bt 4	1.6417	54.3694	35.0709	48.9756	72.3817
Bt 7	1.5202	50.1006	33.4100	51.7600	66.5681
Bt 12	1.9493	52.6554	27.4565	36.7357	76.0954
SBTI 11	1.8276	60.4385	34.1248	49.7643	69.0388
SBTI 12	2.2435	48.5656	26.1133	36.7589	75.3298
Control	3.4388	61.7744	19.2222	24.5422	79.9701
SE±	0.3	3.5	3.1	5.8	3.8
LSD (5%)	0.9	10.2	9.0	16.8	11.0
CV%	35.1	14.7	24.1	31.1	12.0

Table 10. Food consumption and weight gain by the third-instar larvae of *H. armigera* on transgenic and control plants when reared on transgenic tobacco plants for 3 days (wet weight basis) (ICRISAT, Patancheru, 2000).

Treatment	Mass of food offered		Mass of unconsumed food		Mass of consumed food		Corrected mass of food consumed		Initial mass of larvae		Mass of larvae after feeding period		Larval mass gain		Mass of feaces produced	
	(g)		(g)		(g)		(g)		(g)		(g)		(g)		(g)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Bt 1	2.6780	2.4216	2.6780	2.4216	0.0512	0.0455	0.0512	0.0455	0.0017	0.0012	0.0094	0.0094	0.0077	0.0082	0.0164	0.0131
Bt 4	4.5714	1.5004	4.5714	1.5004	0.2123	0.2380	0.2123	0.2380	0.0047	0.0026	0.0376	0.0467	0.0329	0.0440	0.0548	0.0607
Bt 7	4.3788	1.9118	4.3788	1.9118	0.1549	0.2294	0.1549	0.2294	0.0022	0.0036	0.0264	0.0284	0.0243	0.0314	0.0413	0.0443
Bt 12	4.6993	2.0349	4.6993	2.0349	0.0375	0.2183	0.0375	0.2183	0.0014	0.0011	0.0114	0.0290	0.0101	0.0279	0.0160	0.0291
SBTI 11	3.6462	1.2789	3.6462	1.2789	0.1432	0.0763	0.1432	0.0763	0.0039	0.0024	0.0175	0.0151	0.0133	0.0126	0.0451	0.0189
SBTI 12	2.8649	0.9256	2.8649	0.9256	0.0589	0.0594	0.0589	0.0594	0.0019	0.0021	0.0146	0.0137	0.0124	0.0116	0.0156	0.0134
SE±	0.370		0.370		0.0387		0.0387		0.0004		0.0043		0.0040		0.0083	
LSD (5%)	1.075		1.075		0.1124		0.1124		0.0012		0.0125		0.0116		0.0241	
CV%	29.0		30.3		68.0		68.0		36.6		44.5		44.9		60.4	

Table 11. Consumption and utilization of food by the third-instar larvae of *H. armigera* on transgenic and control plants when reared for 3 days on the transgenic tobacco plants before consumption and utilization studies (wet weight basis) ICRISAT. Patancheru, 2000.

Treatment	Consumption Index (CI)		Growth rate (GR%)		Efficiency of conversion of ingested food (ECI%)		Efficiency of conversion of digested food (ECD%)		Approximate digestibility (AD%)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Bt 1	2.8792	2.9261	44.9724	50.3141	16.2288	18.9473	23.6029	27.0374	68.6734	73.3891
Bt 4	3.3680	3.3520	51.3764	59.5051	16.4951	18.2972	23.2113	25.7551	71.9980	75.4863
Bt 7	3.6541	4.5064	57.2805	69.1824	15.9103	18.0135	21.4501	24.3887	74.2357	78.1178
Bt 12	1.8923	4.3083	51.1037	60.6092	29.8916	18.5305	48.5722	30.3839	62.3213	78.8876
SBTI 11	4.5037	2.7434	36.0404	45.0947	10.4149	17.0251	14.6417	22.8780	70.9147	74.4622
SBTI 12	2.3887	2.4648	47.8936	48.4772	20.3335	19.9337	27.9826	25.9081	73.0914	77.0841
SE±	0.56		3.84		2.98		6.07		4.72	
LSD (5%)	1.63		11.15		8.66		17.63		13.70	
CV%	38.6		16.6		36.4		51.6		14.4	

Table 12. Food consumption, weight gain, and amount of feaces produced by the third-instar larvae of *H. armigera* reared on *Cry 1A(b)* and *SBTI* transgenic tobacco plants (dry weight basis) (ICRISAT, Patancheru, 2000).

Treatment	Mass of food offered (g)	Dry mass of food offered (g)	Mass of unconsumed food (g)	Corrected mass of consumed food (g)	Initial mass of larvae (g)	Initial dry mass of larvae (g)	Dry mass of larvae after feeding period (g)	Larval mass gain (g)	Mass of feaces produced (g)
BT 1	2.6210	0.4417	0.4273	0.0144	0.0025	0.0006	0.0038	0.0032	0.0061
Bt 4	3.1368	0.5430	0.5330	0.0101	0.0021	0.0004	0.0034	0.0029	0.0031
Bt 7	2.5911	0.4397	0.4319	0.0078	0.0035	0.0007	0.0028	0.0021	0.0030
Bt 12	3.5590	0.6058	0.5938	0.0119	0.0037	0.0011	0.0037	0.0025	0.0034
SSTI 11	2.5574	0.4321	0.4193	0.0128	0.0023	0.0007	0.0038	0.0031	0.0044
SSTI 12	2.8521	0.4683	0.4540	0.0144	0.0034	0.0011	0.0040	0.0029	0.0042
Control	2.1378	0.3421	0.3106	0.0315	0.0021	0.0006	0.0057	0.0051	0.0079
SE±	0.3130	0.5240	0.0520	0.0030	0.0003	0.0001	0.0007	0.0007	0.0012
LSD (5%)	0.9001	1.5220	0.1510	0.0087	0.0009	0.0003	0.0020	0.0020	0.0035
CV%	25.2	25.1	25.5	51.6	26.5	24.7	40.8	47.5	58.0

Table 13. Consumption, digestion and utilization of food by the third-instar larvae of *H. armigera* fed on Cry 1A(b) and SBTI transgenic plants (dry weight basis) (ICRISAT, Patancheru, 2000)

Treatment	Consumption index (CI)	Growth rate (GR%)	Efficiency of conversion of ingested food (ECI%)	Efficiency of conversion of digested food (ECD%)	Approximate digestibility (AD%)
BT 1	2.0246	44.1050	22.5727	38.2609	59.5103
Bt 4	1.6776	50.2102	31.7670	46.7812	68.4274
Bt 7	1.5218	38.9788	26.2499	44.1138	60.4655
Bt 12	1.6391	34.7157	21.5304	30.5943	71.3400
SBTI 11	1.7519	44.3720	27.1664	42.8199	64.0065
SBTI 12	1.9858	37.3885	21.2778	32.3118	68.9638
Control	3.3407	53.3449	17.2400	23.3464	74.8104
SE±	0.2740	3.0700	2.7500	4.8100	3.2400
LSD (5%)	0.7959	8.9171	7.9876	13.9711	9.4109
CV%	30.7	15.8	25.6	29.2	10.9

Table 14 : Food consumption and weight gain by the third-instar larvae of *H. armigera* on transgenic and control plants when reared on transgenic plants for 3 days (dry weight basis) (ICRISAT, Patancheru, 2000).

Treatments	Mass of food offered (g)		Dry mass of food offered (g)		Mass of unconsumed food (g)		Mass of consumed food (g)		Initial weight of larvae (g)		Initial dry weight of larvae (g)		Dry mass of larvae after feeding period (g)		Larval weight gain (g)		Mass of feaces produced (g)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Bt 1	2.6780	2.4216	0.4603	0.3948	0.4490	0.3866	0.0113	0.0082	0.0017	0.0012	0.0004	0.0003	0.0019	0.0016	0.0015	0.0013	0.0040	0.0024
Bt 4	4.5714	1.5004	0.8282	0.2769	0.8026	0.2543	0.0256	0.0241	0.0047	0.0026	0.0010	0.0007	0.0044	0.0048	0.0034	0.0040	0.0078	0.0075
Bt 7	4.3788	1.9118	0.7694	0.3426	0.7459	0.2996	0.0235	0.0430	0.0022	0.0036	0.0004	0.0010	0.0024	0.0063	0.0019	0.0053	0.0058	0.0107
Bt 12	4.6993	2.0349	0.8062	0.3606	0.8020	0.3413	0.0043	0.0193	0.0014	0.0011	0.0004	0.0003	0.0014	0.0029	0.0010	0.0026	0.0019	0.0034
Sbti 11	3.6462	1.2789	0.6062	0.2168	0.6101	0.2017	0.0305	0.0151	0.0039	0.0024	0.0012	0.0007	0.0032	0.0031	0.0020	0.0024	0.1010	0.0041
Sbti 12	2.8649	0.9256	0.4905	0.1576	0.4672	0.1495	0.0132	0.0082	0.0019	0.0021	0.0006	0.0006	0.0032	0.0020	0.0026	0.0014	0.0034	0.0023
SE±	0.370		0.060		0.062		0.0040		0.0004		0.0001		0.0005		0.0005		0.0010	
LSD (5%)	1.075		0.180		0.179		0.0120		0.0012		0.0003		0.0020		0.0010		0.0040	
CV%	29.0		29.1		30.6		49.2		36.6		35.4		38.0		45.2		52.1	

Table 15 : Consumption and utilization of food by the third-instar larvae of *H. armigera* on transgenic and control plants when reared for 3 days on the transgenic plants before consumption and utilization studies (dry weight basis) (ISRISAT, Patancheru, 2000)

Treatments	Consumption Index (CI)		Growth rate (GR*.)		Efficiency of conversion of ingested food (ECI*.)		Efficiency of conversion of digested food (ECD*.)		Approximate digestability (AD%)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Bt 1	3.0433	2.8411	42.5956	43.9064	14.4834	17.3111	22.0488	25.7904	65.4927	70.3053
Bt 4	3.2752	3.0785	42.3616	47.2537	14.0009	15.7834	20.6487	23.4266	68.6154	70.9657
Bt 7	6.0749	4.0095	46.5310	47.6552	8.0447	14.6925	10.7750	21.3436	76.3495	73.0393
Bt 12	1.5096	4.1335	35.8351	52.6491	26.0493	17.4088	45.4387	29.2756	58.6889	76.9554
Sbti 11	4.1504	2.6016	28.8966	39.0798	9.0971	15.5493	13.3461	21.4675	68.2149	72.3507
Sbti 12	2.2147	2.1724	43.1078	35.5377	19.5053	16.8199	26.7051	23.2528	73.6354	72.2782
SE±	0.55		3.02		2.84		6.03		4.42	
LSD (5%)	1.58		8.62		8.11		17.22		12.63	
CV%	38.0		16.0		40.4		57.1		14.0	

Larval development

The larval duration varied from 17.9 days in larvae reared on 723 Bt 7 to 19.4 days in those reared on 723 Bt 1 plants (Table 16). On untransformed plants, larval duration was 18.6 days. Larval duration was more in larvae reared on 723 Bt 1 plants.

Percentage pupation

Percentage pupation varied from 16% in larvae reared on 737 SBTI 11 plants to 80% on those reared on untransformed plants (Figure 4). Pupation percentage was lower in larvae reared on 723 Bt 4 plants, followed by those reared on 737 SBTI 12 plants. Similarly, low pupation percentage was also recorded in larvae reared on 737 SBTI 12, 723 Bt 1, 723 Bt 7, 737 SBTI 1, and 723 Bt 12 plants as compared to those reared on control plants.

Adult emergence

Percentage adult emergence (of the total number of larvae released) varied from 0 to ~~75~~ 60%. No adult emergence was observed in larvae reared on 737 SBTI 11 plants (Figure 5). Low adult emergence was recorded in larvae reared on 737 SBTI 12, 723 Bt 4, 737 SBTI 1, 723 Bt 1, 723 Bt 7 and 723 Bt 12 plants, as compared to those reared on control plants (~~75~~ 60%).

Fig. 3. Larval mortality of *H. armigera* on different transgenic lines of tobacco

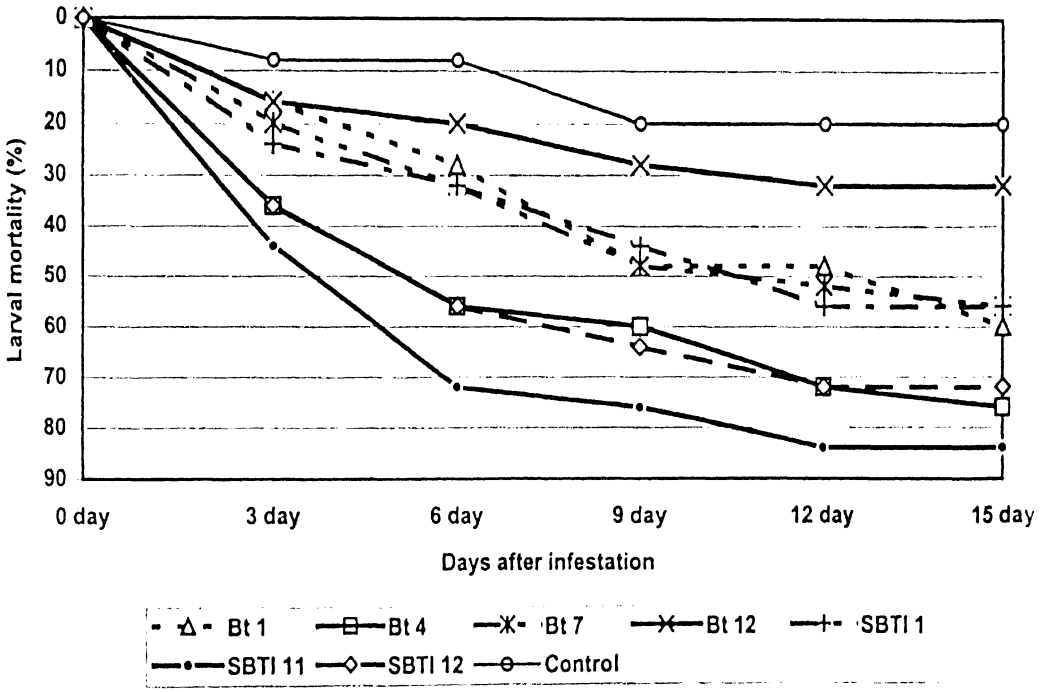


Fig.4. Pupation of *H. armigera* on Bt and SBTI transgenic tobaccos (ICRISAT, Patancheru, 2000)

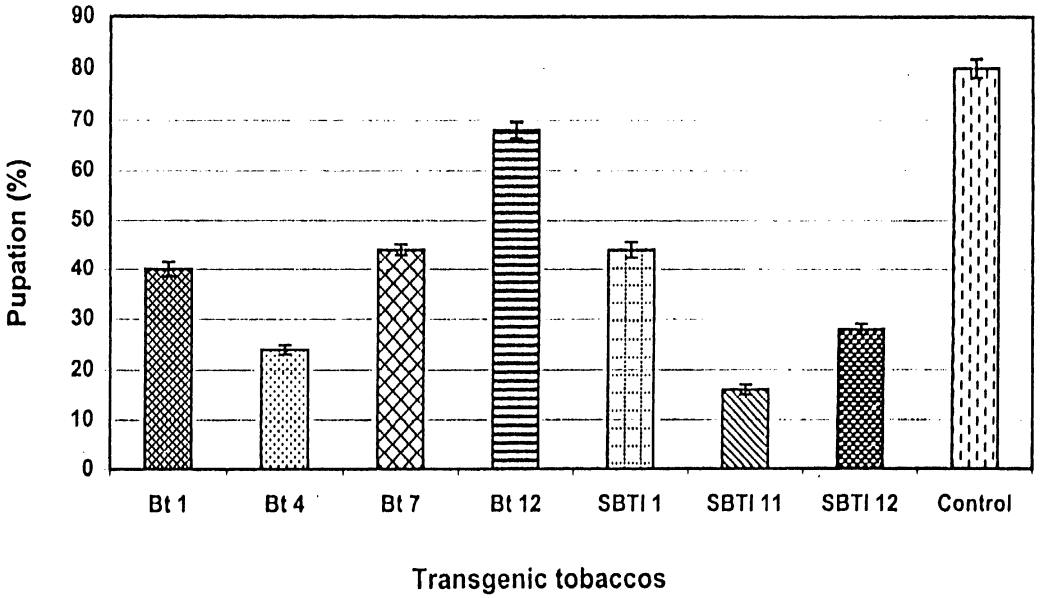


Fig.5. Adult emergence of *H. armigera* on Bt and SBTI transgenic tobaccos (ICRISAT, Patancheru, 2000)

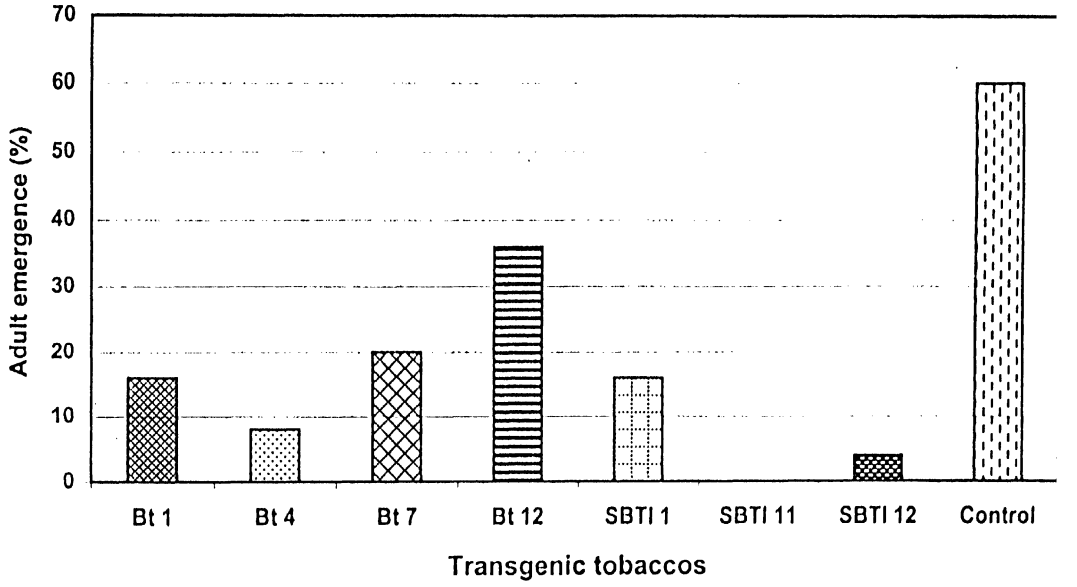


Table 16. Duration of larval period of *H. armigera* on Bt and SBTI transgenic tobaccos (ICRISAT, Patancheru, 2000)

Treatment	Larval duration (days)	Range (days)
723 Bt1	19.4 ±1.5	19 - 20
723 Bt4	19.3 ±0.96	19 - 20
723 Bt7	17.9 ±1.11	17 - 20
723 Bt 12	18.9 ±1.55	18 - 20
737 SBTI 1	18.5 ±1.60	18 - 19
737 SBTI 11	19.0 ±1.0	19 - 19
737 SBTI 12	19.1 ±1.03	18 - 20
Control	18.6 ±1.78	17 - 20

CHAPTER V

DISCUSSION

DISCUSSION

Studies were carried out under greenhouse as well as in laboratory conditions for evaluating eleven independent tobacco transformants of Bt and SBTI carrying Bt Cry I A (b) and SBTI genes, Seven plant lectins and Soybean trypsin inhibitor (Kunitz) for antifeedant, food consumption and utilization and antibiotic studies against legume pod borer *Helicoverpa armigera* Hubner. The parameters investigated included antifeedant activity of Bt and SBTI genes against first and third-instar larvae of *H. armigera* under no - choice tests and dual-choice test, effect of Bt and SBTI genes on food consumption and utilization by third-instar larvae of *H. armigera*, and antibiosis in terms of larval survival, larval mass, pupal mass, and percentage pupation and adult emergence.

Antifeedant activity

In the no-choice tests, antifeedant activity of the transgenic tobacco plants was evident both towards the first- and the third-instar larvae. Studies on antifeedant activity of the putative transformed plants towards *H. armigera* indicated that 723 Bt 1, 723 Bt 4, 723 Bt 7, and 737 SBTI 5 reduced the leaf feed significantly over the nontransformed control. There was a significant reduction in leaf disc consumption by the third-instar larvae on 723 Bt 4, 723 Bt 1, 723 Bt 7, 723 Bt 8, 723 Bt 10 and 737 SBTI 5 as compared to the non-transformed control (2.07 cm^2 unconsumed leaf disc area). Mortality of the first-instar larvae was higher when fed on 723 Bt 1, 723 Bt 4, 723 Bt 7, 723 Bt 10, and 737 SBTI 5 than those fed on the control plants. Larval weight was significantly lower in the larvae fed on 723 Bt 1, 723 Bt 4, 723 Bt 7, 723 Bt 8, and 737 SBTI 5 (1.4 to 2.7 mg per larvae) as compared to those reared on the non-transformed plants (9.3 mg per larvae).

In dual-choice tests, the feeding by the third-instar larvae of *H. armigera* was significantly greater on the control discs than those of the transformed plants of 723 Bt 1, 723 Bt 8, 723 Bt 10, 723 Bt 12, 737 SBTI 5, 737 SBTI 11 and 737 SBTI 12 at 24 and 48 h after initiating the experiment. There was a considerable reduction in leaf feeding by the first-instar and the third-instar larvae when fed on the putative transgenic tobacco plants containing CryIA(c) and the SBTI genes, and such effects were more apparent under the dual-choice than under no-choice conditions. Further, the adverse effects of the transgenic plants were also evident in terms of reduced larval weights, and insect survival. There was some variation in different transgenic lines in terms of expression of resistance to the first- and the third-instar larvae of *H. armigera*.

Percentage larval mortality varied from 20% on untransformed control plants to 84% on the transformed plants. Larval mortality was more than 50% on 723 Bt 1, 723 Bt 7, and 737 SBTI 1 transformed lines. Larval duration varied from 17.9 days in larvae reared on 723 Bt 7 to 19.4 days in those reared on 723 Bt 1 plants. Percentage pupation varied from 16% in larvae reared on 737 SBTI 11 plants to 80% on those reared on untransformed plants. Pupation percentage was lower in larvae reared on 723 Bt 4 plants, followed by those reared on 737 SBTI 12 plants. Percentage adult emergence varied from 0 to 75%. No adult emergence was observed in larvae reared on 737 SBTI 11 plants. Low adult emergence was recorded in larvae reared on 737 SBTI 12, 723 Bt 4, 737 SBTI 1, 723 Bt 1, 723 Bt 7 and 723 Bt 12 plants, as compared to those reared on control plants (75%). Thus, putative transgenic plants expressed both antifeedant and antibiotic effects against the first- and the third-instar larvae of *H. armigera*. Such adverse effects varied across insect stage, and different transgenic lines.

Biological activity of Bt and SBTI genes expressed in different plant species has been demonstrated earlier by several workers. In the present studies to use tobacco for adapting the transformation protocols also confirmed the

activity of these genes against *H. armigera*, the major pest of several field crops in Asia and Africa. Hoffmann *et al.* (1992) reported that the mortality of the larvae was high and the leaf damage was low for the genotypes contaning Bt gene as compared to control and CpTI genotype. Transgenic tobacco contaning *B. thuringiensis* (Bt) and cowpea trypsin inhibitor (CpTI) genes showed insecticidal activity towards *H. armigera* (Zhao *et al.*, 1997). Mortality of the larvae was low on transgenic tobacco expressing Bt alone than the plants expressing both Bt and CpTI. It was concluded that gene pyramiding could be a valuable strategy for resistance management and the sustainable use of Bt transgenic crops. Transgenic tobacco plants containing Bt or CpTI or both of the genes proved toxic to larvae of *H. armigera*. Plants with both transgenes had enhanced resistance compared to those with single transgenes (Zhao *et al.*, 1996).

Soybean trypsin inhibitor (STI) containing transgenic tobacco plants showed high resistance to the larvae of *H. armigera*. (Gao *et al.*, 1998). Li *et al.* (1998) obtained transgenic cotton lines contaning Cowpea trypsin inhibitor (CpTI) gene and found them to be highly resistant to cotton bollworm. Transgenic rice plants contaning Soybean Kunitz trypsin inhibitor (SKTI) showed resistance to the brown planthopper *(Nilaparvata lugens Stal)* (Lee *et al.*1999). Transgenic poplar plants expressing a Kunitz proteinase inhibitor (Kti3) gene did not affect larval mortality, growth and pupal weights of *Lymantria dispar* and *Clostera anastomosis* (Confalonieri *et al.*, 1998). Proteinase inhibitors from *Nicotiana alata* in artificial diet significantly reduced the growth of the native budworm larvae (*H. punctigera*) and the black field cricket nymphs *(Teleogryllus commodus)*. When expressed in transgenic tobacco, these inhibitors showed significant differences in mortality and growth rate of *H. punctigera* larvae. (Heath *et al.*, 1997).

Bioassay of SBTI and plant lectins against *H. armigera*

SBTI (49%), and lectins from snowdrop (64%), and chickpea (65%) influenced

the development and survival of H. armigera larvae, while Concanavalin A, lentil, soybean, and peanut lectins did not affect the survival of *H. armigera* larvae. Lectins did not affect the weight gain of the larvae. Pupal mass varied from 272.6 mg in larvae reared on diet having chickpea lectin to 359.2 mg in larvae reared on diet having snowdrop lectin. Low pupal mass was recorded in larvae reared on diet having chickpea lectin (272.6 mg) as compared to those reared on the control diet (335.4 mg). In snowdrop and peanut lectins, the pupal mass was more as compared to the larvae reared on the control diet. Percentage pupation was lowest in larvae reared on diet impregnated with SBTI (40%), followed by those reared on diets containing chickpea (50%), snowdrop (60%) and soybean lectins (70%). Adult emergence was lowest in larvae reared on diet having snowdrop lectin (20%), followed by those reared on SBTI (30%) and chickpea lectin (30%) as compared to the untreated control (90%).

Adverse biological effects of protease inhibitors have been demonstrated against European corn borer, *Ostrinia nubilalis* (Steffens *et al.*, 1978), *M. sexta* (Shukle *et al.*, 1983), *H. zea* and *S. exigua* (Broadway and Duffey (1986), *H. armigera* (Johnston *et al.*, 1993; Wang *et al.*, 1996), *S. litura* (McManus *et al.*, 1995), *Cydia pomonella* (Markwick *et al.*, 1995), *Chrysodeixis eriosma* (McManus *et al.*, 1994). However, no differences were observed in the growth rates of *S. litura* or *Thysanoplusia orichalcea* larvae fed on leaves from transgenic or non-transgenic plants (McManus *et al.*, 1994).

Lectins from several plants have diverse effects against the insects belonging to different insect orders. Murdock *et al.* (1990) showed that lectins from osage orange (*Maclura pomifera*), peanut (*Arachis hypogaea*), potato (*Solanum tuberosum*), jimson weed (*Datura stramonium*), and wheat germ (*Triticum aestivum*) delayed the developmental time of *C. maculatus* at dietary levels of 0.2 and 1.0% (w/w). Huesing *et al.* (1991a) showed that wheat germ isolectins were equally effective against the cowpea weevil. N-

acetylglucosamine (G/c NAC) binding lectins from *Oryza sativa* (rice) and *Urtica dioica* (Stinging nettle) increased mortality and development time when fed to the cowpea weevil (Huesing *et al.*, 1991b). Lectins from *Allium sativum* (garlic) and *Galanthus nivalis* (snowdrop) affected the survival of the cowpea weevil larvae (Gatehouse *et al.*, 1992). Of the twenty-six plant lectins tested, WGA, *Bauhinia purpurea agglutinin* (BPA) and the lectin from *Ricinus communis* were toxic to *Ostrinia nubilalis*. Lectins from castor bean, pokeweed (*Phytolaca americana* L.), and the green marine alga, *Codium fragile* (Surinagar) were toxic to the neonate southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Czapla and Lang, 1990). Wheat germ, jackfruit, pea, lentil, red kidney bean, and horse gram reduced the survival of potato leafhoppers, when incorporated into the artificial diet at concentration of 0.2 to 1.5% (w/v) (Habibi *et al.*, 1993). Lectins GNA and WGA reduced honeydew excretion levels of adult rice brown planthopper, when added to artificial diet at 0.1% (w/v) (Powell *et al.*, 1995). Shukle and Murdock (1983) reported that soybean lectin inhibited larval growth of *Manduca sexta*, when incorporated into an artificial diet at a 1% level. GNA reduced weight gain and survival when bioassayed against *Spodoptera littoralis* at the 5% level (Gatehouse *et al.*, 1992).

Consumption, digestion and utilization of food

Larvae consumed more food per unit of body mass (CI) and gained more weight when reared on the control plants compared to those reared on transformed plants. Except 737 SBTI 11, growth rate (GR) was significantly more in the larvae reared on control plants compared to those reared on the transformed plants. Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) into body matter was significantly greater in larvae fed on transformed plants in comparison to those fed on the control plants. Approximate digestibility was significantly low in the larvae fed on transformed plants as compared to those fed on control

plants. Mass of food consumed by the third-instar larvae of *H. armigera* was significantly greater in larvae reared on 737 SBTI 11 as compared to those fed on control plants, whereas in case of larvae reared on 723 Bt 12 and 723 Bt 7, the mass of food consumed was significantly lower on the transgenic plants as compared to the control plants. Weight gain by larvae was significantly lower in larvae reared on 723 Bt 12, 723 Bt 4 and 723 Bt 7 as compared to the controls, whereas in case of larvae reared on 737 SBTI 12 and 737 SBTI 11, the mass of larvae was more as compared to that on the control plants. On 737 SBTI 11, larvae consumed more food per unit of body mass (CI) compared to the control plants. Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) was significantly higher in case of larvae reared on 723 Bt 12. On 737 SBTI 11 the ECI and ECD values were significantly lower in larvae reared on these lines as compared to those fed on the control plants. Approximate digestibility (AD) was significantly lower in case of larvae fed on 723 Bt 12 plants as compared to those fed on the control plants. This suggests that the more consumption of food increased the body size as well as the development of the larvae. These studies were in consonance with studies made on *Trichoplusia ni* reared on soybean leaves, where there is differential response in the growth of the fast and slow growing larvae in proportion to the consumption as well as the utilization of food (Sharma and Norris, 1993).

CHAPTER VI

SUMMARY, CONCLUSION

&

UGGESTIONS FOR FURTHER WORK

SUMMARY

Antifeedant and the food consumption and utilization studies against the legume pod borer, *Helicoverpa armigera* were conducted using eleven independent transgenic tobacco lines (723 Bt 1, 723 Bt 3, 723 Bt 4, 723 Bt 7, 723 Bt 8, 723 Bt 10, 723 Bt 12, 737 SBTI 1, 737 SBTI 5, 737 SBTI 11, 737 SBTI 12 and nontransformed Control) transformed with Bt Cry 1A(b) and SBTI genes under laboratory conditions. Antibiotic effects in terms of larval survival, larval mass, pupal mass, and percentage pupation and adult emergence were studied under laboratory conditions using different plant lectins (concanavalin A, lentil, jacalin, soybean, peanut, snowdrop and chickpea lectins) and soybean trypsin inhibitor (SBTI) in artificial diet. Effects of transgenic tobacco plants on the biology of *H. armigera* were examined in terms of larval survival, larval duration, and percentage pupation and adult emergence under laboratory conditions using seven transgenic tobacco lines.

In no-choice tests with first-instar larvae; 723 Bt 1, 723 Bt 4, 723 Bt 3, 723 Bt 7, 737 SBTI 5 and 737 SBTI 1 reduced the leaf feeding significantly over the nontransformed control. However, in no-choice tests with third-instar larvae, the leaf disc consumption was significantly lower on 723 Bt 4, 723 Bt 1, 723 Bt 7, 723 Bt 8, 723 Bt 10 and 737 SBTI 5 as compared to the control. In dual-choice tests, the feeding by the third-instar larvae of *H. armigera* was significantly greater on the control leaf discs than on the transformed plants of 723 Bt 1, 723 Bt 8, 723 Bt 10, 723 Bt 12, 737 SBTI 5, 737 SBTI 11 and 737 SBTI 12.

Antibiotic effects of different plant lectins and soybean trypsin inhibitor were determined by incorporating these chemicals into the artificial diet (0.1% concentration). Of the seven lectins tested, chickpea and snowdrop lectins showed marked antibiotic effects towards *H. armigera* larvae. Larval survival was lower on soybean trypsin inhibitor, snowdrop lectin, and chickpea lectin treated diets (49, 64 and 65%, respectively) at five days after infestation.

Larval mass was considerably lower for insects reared on control diet compared to other treatments. Lectins did not affect the weight gain of the larvae. Pupal mass was adversely affected by chickpea lectin. Lower pupation was observed in diets impregnated with soybean trypsin inhibitor, chickpea lectin, and snowdrop lectin (40, 50 and 60% respectively). Lower adult emergence was observed in diets treated with snowdrop lectin, chickpea lectin, and soybean trypsin inhibitor.

Food consumption and utilization by third-instar larvae of *H. armigera* indicated that on control plants, the larvae consumed more food per unit of body mass (CI), and gained more weight than on the transformed plants. Growth rate (GR) of larvae was more on control plants than on transformed plants. Efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) into body matter was higher on transformed plants than on control plants. Approximate digestibility (AD) was more in larvae fed on control plants than on transformed plants. Consumption and utilization of food of larvae reared on transgenic lines indicated that on 737 SBTI 11, the CI was more compared to control plants, whereas on 723 Bt 12 and 723 Bt 7, the CI was lower as compared to the control. Except on 737 SBTI 12, the GR was more on control plants as compared to that on the transgenic lines. ECI and ECD were high on 723 Bt 12, whereas on 737 SBTI 11, the ECI and ECD were significantly lower compared to the control plants. AD was significantly lower in larvae fed on 723 Bt 12 plants compared to control plants.

Studies on the effects of transgenic tobacco plants on the biology of *H. armigera* revealed that larval mortality was higher on 737 SBTI 11, 723 Bt 4 and 737 SBTI 12 transformed plants (84, 76 and 72% mortality, respectively). Larval mortality was more than 50% on 723 Bt 1, 723 Bt 7, and 737 SBTI 1 transformed lines. The larval duration varied from 17.9 in larvae reared on 723 Bt 7 to 19.4 days in those reared on 723 Bt 1 plants. On control plants larval duration was 18.6 days. Lower pupation was observed in larvae reared on

737 SBTI 11, 723 Bt 4 and 737 SBTI 12 plants. Lower adult emergence was seen on 737 SBTI 12, 723 Bt 4, 723 Bt 1 and 737 SBTI 1 plants. No adult emergence was observed in larvae reared on 737 SBTI 11 plants.

CONCLUSION

Among the eleven transgenic tobacco lines tested, line Bt 1, Bt 4, Bt 7, Bt 12, SBTI 5, SBTI 11 and SBTI 12 were found to be most effective in reducing the damage caused by *H. armigera*. Among the lectins tested larval survival reduced significantly in snowdrop and Chickpea lectins, when tested at 0.1% concentration.

SUGGESTION FOR FURTHER WORK

1. Field trails should be conducted using transgenic tobacco lines containing Bacillus thuringiensis and Soybean trypsin inhibitor (SBTI) genes for testing the efficacy of these transgenic plants under field conditions.

2. Succcessive generations of transgenic tobacco lines containing Bt and SBTI genes should be tested in order to check any detoriation in the transgenic activity due to segregation.

3. The chemicals of SBTI and plant lectins in the present study were tested at 0.1% concentration. This was the least effective concentration. These chemicals can be tested at different concentrations and their effect on the life cycly of *H. armigera* can be studied.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adamczyk, J. J. Jr., Church, G. E., Holloway, J. W., Graves, B. and Leonard, B. R. (1998). Larval survival and development of the fall armyworm on normal and transgenic cotton possessing the *Bacillus thuringiensis* Cry1A(c) delta endotoxin. *Journal of Economic Entomology* 91: 539-545.

Alcock, B. and Twine, P. H. (1981). The cost of *Heliothis* in Queensland crops. Presented at the workshop on the Biological Control of *Heliothis* spp., 23-25 September 1980. Queensland Department of Primary Industries, Toowoomba, Queensland, Australia. pp.1-10.

Applebaum, S. W. (1985). Biochemistry of digestion. In Comprehensive Insect Physiology, Biochemistry and Pharmacology (eds., G. A. Kerkut, and L. I. Gilbert). Pergamon Press, New York, USA. pp 279-311.

Applebaum, S. W. and Konijn, A. M. (1966). The presence of a *Tribolium*-protease inhibitor in wheat. *Journal of Insect Physiology* 12: 665-669.

Armstrong, C. L., Parker, G. B., Pershing, J. C., Brown, S. M., Sanders, P. R., Duncan, D. R., Stone, T., Dean, D. A., DeBoer, D. L., Hart, J., Howe, A. R., Morrish, F. M., Pajeau, M. E., Peterse, W. L., Reich, B. J., Rodriguez, R., Santino, C. G., Sato, S. J., Schuler, W., Sims, S. R., Stehling, S., Tarochione, L. J. and Fromm, M. E. (1995). Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Science 35*: 550-557.

Aronson, A.I., Beckman, W. and Dunn, P. (1986). *Bacillus thuringiensis* and related insect pathogens. *Microbiology Review* 50: 1-24. δδ

Barton, K. A., Whiteley, H. R. and Yang, N. S. (1987). *Bacillus thuringiensis* δ-endotoxin in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiology* 85: 1103-1109.

Benedict, J. H., Sachs, E. S., Altman, D. W., Deaton, D. R., Kohel, R. J., Ring, D. R. and Berberich, B. A. (1996). Field performance of cotton expressing Cry IA insecticidal crystal protein for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera : Noctuidae). *Journal of Economic Entomology* 89: 230-238.

Berliner, E. (1911). Uber die Schalffsuchi der Mehlmottenraupo (Ephestia Kuhniella Zell.) und thren Erreger, *Bacillus thuringiensis* n. sp. *Zietschfit fur Angewandte Entomologie* 2: 29-56.

Bhatnagar, V. S. (1980). A report on research on *Heliothis* complex at ICRISAT (India) 1974-79. Proceedings of All India Workshop on Consolidation of Pest Management. Recommendations and Guidelines of Research, 24-26 April, 1980, Udaipur, India.

Bhatnagar, V. S., Lateef, S. S., Sithanantham, S., Pawar, C. S. and Reed, W. (1982). Research on *Heliothis* at ICRISAT. Proceedings of the International Workshop on *Heliothis* Management (eds. W. Reed, and V. Kumble). International Crops Research Institute for the Semi-arid Tropics, Patancheru, Andhra Pradesh, India. pp 385-396.

Birk, Y., Gertler, A. and Khalef, S. (1963). Separation of *Tribolium*-protease inhibitor from soybeans on a calcium phosphate column. *Biochemistry and Biophysica Acta* 67: 326-328.

Birk,Y. and Applebaum, S. W. (1960). Effect of soybean trypsin inhibitors on the development and midgut proteolytic activity of *Tribolium castaneum* larvae. *Enzymologia* 22: 318-326.

Boyd, W. C. and Reguera, R. M. (1949). Studies on haemagglutinins present in seeds of some representatives of the family Leguminoseae. *Journal of Immunology 62*: 333-339.

Broadway, R. M. (1989). Characterization and ecological implications of midgut proteolytic activity in larval *Pieris rapae* and *Trichoplusia ni*. *Journal of Chemical Ecology 15*: 2101-13.

Broadway, R.M. and Duffey, S. S. (1986). Plant proteinase inhibitors: Mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology* 32: 827-33.

Brown, W. E., Takio, K., Titani, K. and Ryan, C. A. (1985). Wound-induced trypsin inhibitor in alfalfa leaves: identify as a member of the Bowman-Birk inhibitor family. *Biochemistry 24:* 2105-2108.

Carroll, J. and Ellar, D. J. (1993). Proteolytic processing of coleopteran specific δ-endotoxin by *Bacillus thuringiensis* var. *tenebrionis*. *European Journal of Biochemistry 214:* 771-778.

Charles, J. F., Nielsen-Le Roux, C. and Delecluse, A. (1996). *Bacillus sphaericus* toxins : Molecular biology and mode of action. *Annual Review of Entomology* 41: 451-472.

Choma, C. T. and Kaplan, H. (1990). Folding and unfolding of the protoxin from *Bacillus thuringiensis* : evidence that the toxic moiety is present in an active conformation. *Biochemistry* 29: 10971-10977.

Choma, C. T., Surewicz, W. K., Carey, P. R., Pozsgay, M. and Raynor, T. (1990). Unusual proteolysis of the protoxin and toxin from *Bacillus thuringiensis* : Structural implications. *European Journal of Biochemistry* 189: 523-527.

Chrispeels, M.J. and Raikhel, N.V. (1991). Lectins, lectin genes, and their role in plant defense. *The Plant Cell* 3: 1-9.

Confalonieri, M., Allegro, G., Balestrazzi, A., Fogher, C. and Delledonne, M . (1998). Regeneration of *Populus nigra* transgenic plants expressing a Kunitz proteinase inhibitor (KTi3) gene. *Molecular Breeding* 4:137-145.

Convents, D., Houssier, C., Lasters, I. and Lauwereys, M. (1990). The *Bacillus thuringiensis* δ-endotoxin. Evidence for a two domain structure of the minimal toxic fragment. *Journal of Biological Chemistry* 265: 1369-1375.

Cornu, D., Leple, J.C., Bonade-Bottino, M., Ross, A., Augustin, S., Delplanque, A., Jouanin, L. and Pilate, G. (1996). Expression of a proteinase inhibitor and a *Bacillus thuringiensis* δ-endotoxin in transgenic poplars. Proceedings, IUFRO Meeting on Somatic Cell Genetics and Molecular Genetics of Trees. Kluwer, Dordrecht, The Netherlands. pp.131-136.

Czapla, T. H. and Lang, B. A. (1990). Effect of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and southern corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 83: 2480-2485.

Dhandapani, N. and Balasubramanian, M. (1980). Effect of different food plants on the development and reproduction of *Heliothis armigera* (Hubner). *Experientia 36* : 930-931.

Dixon, H. B. F. (1981). Defining a lectin. *Nature* 338: 192.

Elfstrand, M. (1898). *Uber blutkorperchenagglutinierende Eiweisse. In: Gorberdorfer Veroffentlichungen a. Band I.* (Kobert, R., ed.) Enke, Stuttgart, Germany. 159 pp.

Etzler, M. E. (1986). Distribution and function of plant lectins. (eds. Liener, I. E., Sharon, N. and Goldstein, I. J.), The Lectins: Properties, Functions and Applications in Biology and Medicine. New York, USA : Academic Press. pp. 371-435.

Fischhoff, D. A., Bowdish, K. S., Perlak, F. J., Marrone, P. G., McCormick, S. M., Niedermeyer, J. G., Dean, D. A., Kusano-Kretzmer, K., Mayer, E. J., Rochester, D. E., Rogers, S. G. and Fraley, R. T. (1987). Insect tolerant tomato plants. *BioTechnology* 5: 807-813.

Fitt, G. P. (1989). The ecology of *Heliothis* in relation to agroecosystems. *Annual Review of Entomology* 34: 17-52.

Fujimoto, H., Itoh, K., Yamamoto, M., Kayozuka, J. and Shimamoto, K. (1993). Insect resistant rice generated by introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis. BioTechnology* 11: 1151-1155.

Gao, Y.F., Zhu, Z., Xiao, G. F., Zhu, Y., Wu, Q. and Li, X. H. (1998). Isolation of soybean Kunitz trypsin inhibitor gene and its application in plant insect-resistant genetic engineering. *Acta Botanica Sinica*. 40: 405-411.

Garcia-Olmedo, F., Salcedo, G., Sanchez-Monge, R., Gomez, L., Royo, J. and Carbonero, P. (1987). Plant proteinaceous inhibitors of proteinases and -amylases. *Oxford Surveys of Plant Molecular and Cell Biology* 4: 275.

Garczynski, S. F., Crim, J. W. and Adang, M. J. (1991). Identification of a putative brush border membrane-binding molecules specific to *Bacillus thuringiensis* δ-endotoxin by protein blot analysis. *Applied Environmental Microbiology 57*: 2816-2820.

Gatehouse, A. M. R. and Boulter, D. (1983). Assessment of the antimetabolic effect of trypsin inhibitors from cowpea (*Vigna unguiculata*) and other legumes on development of the bruchid beetle, *Callosobruchus maculatus. Journal of Science and Food Agriculture 34*: 345-50.

Gatehouse, A. M. R., Dewey, F. M., Dove, J., Fenton, K. A. and Pusztai, A. (1984). Effect of seed lectins from *Phaseolus vulgaris* on the development of larvae of *Callosobruchus maculatus*: mechanism of toxicity. *Journal of Science and Food Agriculture* 35: 373-380.

Gatehouse, A. M. R., Gatehouse, J. A., Dobie, P., Kilminster, A. M. and Boulter, D. (1979). Biochemical basis of insect resistance in *Vigna unguiculata*. *Journal of Science and Food Agriculture 30*: 948-958.

Gatehouse, A. M. R., Powell, K. S., Van Damme, E. J. M. and Gatehouse, J. A. (1995). Insecticidal properties of plant lectins. Lectins, Biomedical Perspectives (eds., A.Pusztai and S.Bardocz). London, UK : Taylor and Francis.

Gatehouse, A., Hilder, V., Van Damme, E., Peumans, W., Newell, C. and Hamilton, W. (1992). Insecticidal proteins, World Intellectual Patent Organisation Application, No. WO 92/02139.

Gill, S. S., Cowles, E. A. and Pietrantonio, F. V. (1992): The mode of action of *Bacillus thuringiensis* endotoxins. *Annual Review of Entomology* 37: 615-636.

Goldberg, L. J. and Mangalit, J. (1977). A bacterial spore demonstrating rapid larvicidal activity against *Anopheles serengetii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens*. *Mosquito Newsletter 37*: 355-358.

Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T. and Sharon, N. (1980). What should be called a lectin? *Nature 285:* 66.

Green, T. R. and Ryan, C. A. (1972). Wound-induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. *Science* 175: 776-777.

Habibi, J., Backus, E. A. and Czapla, T. H. (1993). Plant lectins affect survival of the potato leafhopper (Homoptera: Cicadellidae). *Journal of Economic Entomology* 86: 945-951.

Hannay, C. L. (1953). Crystalline inclusions in aerobic spore-forming bacteria. *Nature* 172: 1004.

Hannay, C. L. and Fitz-James, P. (1955). The protein crystals of *Bacillus thuringiensis* Berliner. *Canadian Journal of Microbiology* 1: 674-710.

Heath, R. L., McDonald, G., Christeller, J. T., Lee, M., Bateman, K., West, J., Van-Heeswijck, R. and Anderson, M.A. (1997). Proteinase inhibitors from *Nicotiana alata* enhance plant resistance to insect pests. *Journal of Insect Physiology* 43: 833-842.

Hendrickx, K., DeLoof, A. and Van Mellaert, H. (1989). Effects of *Bacillus thuringiensis* δ-endotoxins on the permeability of brush border membrane vesicles from tobacco hornworm (*Manduca sexta*) midgut. *Comparative Biochemistry and Physiology 95*(C): 241-245.

Hilder, V. A. and Boulter, D. (1999). Genetic engineering of crop plants for insect resistance - a critical review. *Crop Protection 18*: 177-191.

Hilder, V. A., Gatehouse, A. M. R., Powell, K. S. and Boulter, D. (1993). Proteins with insecticidal properties against homopteran insects and their use in plant protection. *World Intellectual Patent Organization Application No. WO 93/04177.*

Hilder, V. A., Gatehouse, A. M. R., Sheerman, S. E., Baker, R. F. and Boulter, D. (1987). A novel mechanism of insect resistance engineered into tobacco. *Nature* 330: 160-163 .

Hoffman, M. P., Zalom, F. G., Wilson, L. T., Smilanick, J. M., Malyj, L. D., Kiser, J., Hilder, V. A. and Barnes, W. M. (1992). Field evaluation of transgenic tobacco containing genes encoding *Bacillus thruingiensis* δ-endotoxin or cowpea trypsin inhibitor: Efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 85: 2516-2522.

Hoftey, H. and Whiteley, H. R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis. Microbiology Review* 53: 242-255.

Houseman, J. G., Downe, A. E. R. and Philogene, B. J. R. (1989). Partial characterization of proteinase activity in the larval midgut of the European corn borer *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae). *Canadian Journal of Zoology 67*: 864-68.

Huesing, J. E., Murdock, L. L. and Shade, R. E. (1991a). Effect of wheat germ isolectins on development of cowpea weevil. *Phytochemistry* 30: 785-788.

Huesing, J. E., Murdock, L. L. and Shade, R. E. (1991b). Rice and stinging nettle lectins: insecticidal activity similar to wheat germ agglutinin. *Phytochemistry* 30: 3565-3568.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (1992). The Medium Term Plan, Vol.1. Patancheru, Andhra Pradesh, India : ICRISAT.

Ishiwata, S. (1901). On a kind of severe flasherie (Sotto disease). *Dainihan Sanbshi Kaiho 9*: 1-5.

James, C. (1997). Global status of transgenic crops in 1997. *Abstract of ISAAA Briefs*, No. 5.

Janzen, D. H., Juster, H. B. and Liener, I. E. (1976). Insecticidal action of the phytohemagglutinin in black beans on a bruchid beetle. *Science* 192: 795-796.

Johnson, R., Narvaez, J., An, G. and Ryan, C. A. (1989). Expression of proteinase inhibitors I and II in transgenic tobacco plants: Effects on natural defense against *Manduca sexta* larvae. *Proceedings of the National Academy of Sciences USA*. 86: 9871-9875.

Johnston, K. A., Gatehouse, J. A. and Anstee, J. H. (1993). Effects of soybean protease inhibitors on the growth and development of larval *Helicoverpa armigera*. *Journal of Insect Physiology* 39: 657-664.

Knowles, B. H. and Dow, J. A. T. (1993). The crystal δ-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut. *Bio-Essays 15*: 469-476.

Knowles, B.H., Knight, P. J. K. and Ellar, D. J. (1991). N-acetyl galactosamine is part of receptor in insect midgut epithelium that recognises an insecticidal protein from *Bacillus thuringiensis*. *Proceedings of the Royal Society of London* 245: 31-35.

Koziel, M. G., Beland, G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M. R., Merlin, E., Rhodes, R., Warren, G. W., Wright, M. and Evola, S. V. (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *BioTechnology 11*: 194-200.

Krieg, A., Huger, A. M., Langenbruch, G. A. and Schnetter, W. (1983). *Bacillus thuringiensis* var. *tenebrions* ein newer gegenuber Larven von Coleopteren Wirksan Pathotyp. *Journal of Applied Entomology* 96: 500-508.

Kunitz, M. (1947). Crystalline soybean trypsin inhibitor. 2. General properties. *Journal of General Physiology 30*: 291.

Lambert, B. and Peferoen, M. (1992). Insecticidal promise of *Bacillus thuringiensis*. *Bioscience* 42: 112-121.

Landsteiner, K. and Raubitschek, H. (1907). Beobachtungen Uber Hamolyse und Hamagglutination. *Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg.* Abt. 1: Orig. 45: 660-667.

Laskowski, M. Jr. and Kato, I. (1980). Protein inhibitors of proteinases. *Annual Review of Biochemistry* 49: 593.

Lee, M.K., Milne, R.E., Ge, A. Z. and Dean, D. H. (1992). Location of *Bombyx mori* binding receptor on *Bacillus thuringiensis* δ-endotoxin. *Journal of*

Biological Chemistry 267: 3115-3121.

Lee, S. I., Lee, S.H., Koo, J. C., Chun, H. J., Lim, C.O., Mun, J. H., Song, Y. H. and Cho, M.J. (1999). Soybean Kunitz trypsin inhibitor (SKTI) confers resistance to the brown planthopper (*Nilaparvata lugens* Stal) in transgenic rice. *Molecular Breeding* 5:1-9.

Li,Y.E., Zhu, Z., Chen, Z.X., Wu, X., Wang, W. and Li, S.J. (1998). Obtaining transgenic cotton plants with cowpea trypsin inhibitor. *Acta Gossypii Sinica* 10: 237-243.

Liener, I. and Kakade, M. L. (1969). Protease inhibitor. Toxic Constituents of Plant Foodstuffs (ed., I. E. Liener). New York, USA : Academic Press. pp. 8-66.

Lipke, H., Fraenkel, G. S. and Liener, I. E. (1954). Effect of soybean inhibitors on growth of *Tribolium confusum*. *Journal of Agriculture and Food Chemistry* 2: 410-414.

Manjunath, T. M., Bhatnagar, V. S., Pawar, C. S. and Sithanantham, S. (1989). Economic importance of *Heliothis* spp. in India and an assessment of their natural enemies and host plants. (eds., E.G.King and R.D.Jackson). Proceedings of the Workshop on Biological control of *Heliothis*. Increasing the Effectiveness of Natural Enemies, 11-15 Nov., 1985, New Delhi, India. Far Eastern Regional Research Office, U. S. Department of Agriculture, New Delhi, India. pp. 197-228.

Markwick, N. P., Reid, S. J., Laing, W. A. and Christeller, J. T. (1995). Effects of dietary protein and protease inhibitors on codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology* 88: 33-39.

McCown, B.H., McCabe, D. E., Russell, D. R., Robison, D. J., Barton, K. A. and Raffa, K. F. (1991). Stable transformation of *Populus* and incorporation of pest resistance by electric discharge particle acceleration. *Plant Cell Reports* 9: 590-594.

McLaren, J. S. (1998). The success of transgenic crops in the USA. *Pesticide Outlook* 9: 36-41.

McManus, M. T. and Burgess, E. P. J. (1995). Effects of the soybean (Kunitz) Trypsin inhibitor on growth and digestive proteases of larvae of *Spodoptera litura*. *Journal of Insect Physiology 41*: 731-738.

McManus, M. T., White, D. W. R. and McGregor, P. G. (1994). Accumulation of a chymotrypsin inhibitor in transgenic tobacco can affect the growth of insect pests. *Transgenic Research* 3: 50-58.

Mickel, C. E. and Standish, J. (1947). Susceptibility of processed soy flour and soy grits in storage to attack by *Tribolium castaneum* (Herbst). *Minnesota Agriculture Experiment Station Technical Bulletin* 178: 1-20.

Murdock, L. L., Huesing, J. E., Nielsen, S. S., Pratt, R. C. and Shade, R. E. (1990). Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* 29(1): 85-89.

Oddou, P., Hartmann, H. and Geiser, M. (1991). Identification and characterisation of *Heliothis virescens* midgut membrane proteins binding *Bacillus thuringiensis* δ-endotoxins. *European Journal of Biochemistry* 202: 673-680.

Peferoen, M. (1992). Engineering of insect-resistant plants with *Bacillus thuringiensis* crystal protein genes. Biotechnology in Agriculture No.7: Plant Genetic Manipulation for Crop Protection. London, UK : CAB International. pp.135-153.

Perlak, F. J., Fuchs, R. L., Dean, D. A., McPherson, S. L. and Fischhoff, D. A. (1991). Modification of the coding sequence enhances plant expression of insect control protein genes. *Proceedings of the National Academy of Sciences USA*. 88: 3324-3328.

Perlak, F. J., Deaton, R. W., Armstrong, T. O., Fuchs, R. L., Sims, S. R., Greenplate, J. T. and Fischhoff, D. A. (1990). Insect resistant cotton plants. *BioTechnology* 8: 939-943.

Perlak, F. J., Stone, T. B., Muskopf, Y. N., Petersen, L. J., Parker, G. B., McPherson, S. A., Wyman, J., Love, S., Reed, G., Bięver, D. and Fischhoff, D. A. (1993). Genetically improved potatoes: protection from damage by Colorado potato beetle. *Plant Molecular Biology* 22: 313-321.

Peumans, W. J. and Van Damme, E. J. M. (1995a). Lectins as plant defense proteins. *Plant Physiology* 109: 347-352.

Peumans, W. J. and Van Damme, E. J. M. (1995b). The role of lectins in plant defence. *Histochemistry Journal* 27: 253-271.

Powell, K. S., Gatehouse, A. M. R., Hilder, V. A. and Gatehouse, J. A. (1993). Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*. *Entomologia Experimentalis et Applicata* 66: 119-126.

Powell, K. S., Gatehouse, A. M. R., Hilder, V. A. and Gatehouse, J. A. (1995). Antifeedant effects of plant lectins and an enzyme on the adult stage of the rice brown planthopper, *Nilaparvata lugens*. *Entomologia Experimentalis et Applicata* 75: 51-59.

Powell, K. S., Gatehouse, A. M. R., Hilder, V. A., Van Damme, E. J. M., Peumans, W. J., Boonjawat, J., Horsham, K. and Gatehouse, J. A. (1995). Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens*. *Entomologia Experimentalis et Applicata* 75: 61-65.

Reed, W. and Pawar, C. S. (1982). *Heliothis*: a global problem. Proceedings of the International Workshop on *Heliothis* Management (eds., W.Reed and V.Kumble). International Crops Research Institute for the Semi- arid Tropics, Patancheru, Andhra Pradesh, India. pp. 9-14.

Reed, W., Cardona, C. and Sithanantham, S. (1987). The chickpea insect pests and their control. The Chickpea (eds., M.C.Saxena and K.B.Singh). Abrystwyth, UK : CAB International. pp. 282-318.

Renkonen, K. O. (1948). Studies on hemagglutinins present in seeds of some representatives of leguminoseae. *Annual Medical Experimental Biology* 26: 66-72.

Richardson, M. (1977). The proteinase inhibitors of plants and micro-organisms. *Phytochemistry* 16: 159-169.

Richardson, M. (1980). Protein inhibitors of enzymes. *Journal of Food Chemistry* 6: 235.

Russell, D. (1999). Insecticide resistance and its management in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in India. In: *Proceedings of the 1998 meeting of the Plant Protection Society*, Hyderabad, India.

Ryan, C. A. (1973). Proteolytic enzymes and their inhibitors in plants. *Annual Review of Plant Physiology* 24: 173-196.

Ryan, C. (1990). Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology* 28: 425-449.

Sacchi, V. F., Parenti, P., Giordana, B., Hanozet, G. M., Luthy, P. and Wolfersberger, M. G. (1986). *Bacillus thuringiensis* inhibits K^+ gradient dependent amino acid transport across the brush border membrane of *Pieris brassicae* midgut cells. *FEBS Letters* 204: 213-218.

Santos, M. O., Adang, M. J., All, J. N., Boerma, H. R. and Parrott, W. A. (1997). Testing transgenes for insect resistance using *Arabidopsis*. *Molecular Breeding* 3: 183-194.

Schwartz, J. L., Garneau, L., Masson, L. and Brousseau, R. (1991). Early response of cultured lepidopteran cells to exposure to δ-endotoxin from *Bacillus thuringiensis* improvement of calcium and anionic channels. *Biochemica et Biophysica Acta, Biomembranes 1065*: 250-260.

Shanower, T.G., Romeis, J. and Minja, E. M. (1999). Insect pests of pigeonpea and their management. *Annual Review of Entomology* 44: 77-96.

Sharma, H.C. and Norris, D.M. (1993) Innate differences in consumption and utilization of food by the fast- and slow-growing larvae of cabbage looper, *Trichoplusia ni* (Hubner) (Lep., Noctuidae). *Journal of Applied Entomology* 116: 527-531.

Shukle, R. H. and Murdock, L. L. (1983). Lipoxygenase, trypsin Inhibitor, and lectin from soybeans: Effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). *Enviromental Entomology* 12:787-791.

Shumway, L. K., Yang, V. V. and Ryan, C. A. (1976). Evidence for the presence of proteinase inhibitor I in vacuolar protein bodies of plant cells. *Planta* 129: 161-165.

Sims, S. R. (1995). *Bacillus thuringiensis* var. *kurstaki* (CrylA(c)) protein expressed in transgenic cotton: effects on beneficial and other non-target insects. *Southwestern Entomologist* 20: 493-500.

Singh, H. and Singh, G. (1975). Biological studies on *Heliothis armigera* (Hubner) in Punjab. *Indian Journal of Entomology* 37: 154-164.

Singh, L., Gupta, S. C. and Faris, D. G. (1990). Pigeonpea: breeding. The Pigeonpea (eds. Y.L. Nene, S.D. Hall and V.K. Sheila). Abrystwyth, UK : CAB International. pp. 375-399.

Steffens, R., Fox, F. R. and Kassell, B. (1978). Effect of trypsin inhibitors on growth and metamorphosis of corn borer larvae *Ostrinia nubilalis* (Hubner). *Journal of Agriculture and Food Chemistry* 26:170-174.

Stewart, C. N., Adang, M. J. and All, J. N. (1996a). Genetic transformation, recovery, and characterization of fertile soybean transgenic for a synthetic *Bacillus thuringiensis* CryIA(c) gene. *Plant Physiology* 112: 121-129.

Stewart, C. N., Adang, M. J., All, J. N., Raymer, P. L., Ramachandran, S. and Parrott, W. A. (1996). Insect control and dosage effects in transgenic canola containing a synthetic *Bacillus thuringiensis* CryIA(c) gene. *Plant Physiology* 112: 115-120.

Stillmark, H. (1888). Uber Ricin ein giftiges Ferment aus den Samen von *Ricinus communis* L. und einige anderen Euphorbiaceen. *Inaugural Dissertation Dorpat*, Tartu.

Strizhov, N., Keller, M. and Mathur, J. (1996). A synthetic CryIC gene, encoding a *Bacillus thuringiensis* δ-endotoxin, confers *Spodoptera* resistance in alfalfa and tobacco. *Proceedings of the National Academy of Sciences USA.* 93: 15012-15017.

Strosberg, A. D., Buffard, D., Lauwereys, M. and Foriers, A. (1986). The Lectins: Properties, Functions and Applications in Biology and Medicine. (eds., I. E. Liener, N. Sharon and I. J. Goldstein). New York, USA : Academic Press. pp. 251-263.

Summer, J B. and Howell, S. F. (1936). The identification of the hemagglutinin of the Jack bean with concanavalin A. *Journal of Bacteriology* 32: 227-237.

Tailor, R., Tippett, J., Gibb, G., Pells, S., Pike, D., Jordan, L. and Ely, S. (1992). Identification and characterization of a novel *Bacillus thuringiensis* - endotoxin entomocidal to coleopteran and lepidopteran larvae. *Molecular Microbiology* 7: 1211-1217.

Tripathi, S. R. (1985). Final report of ICAR ad-hoc scheme on "Biology, Food Preference and Extent of Damage by *Heliothis armigera* on Different Varieties of Gram in Tarai Belt of Eastern U.P. Department of Zoology, University of Gorakhpur, U.P., India.

Twine, P. H. (1989). Distribution and economic importance of *Heliothis* (Lepidoptera: Noctuidae) and of their natural enemies and host plants in Australia. Proceedings of the Workshop on Biological Control of *Heliothis* : Increasing the effectiveness of natural enemies, 11-15 November, 1985, New Delhi, India (eds., E. G. King and R. D. Jackson). FERRO, USDA, New Delhi, India. pp. 177-184.

Vaeck, M., Reynaerts, A., Hoftey, H., Jansens, S., DeBeuckleer, M., Dean, C., Zabeau, M., Van Montagu, M. and Leemans, J. (1987). Transgenic plants protected from insect attack. *Nature 327*: 33-37.

Van Parijs, J., Broekaert, W. F., Goldstein, I. J. and Peumans, W. J. (1991). Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. *Planta 183*: 258-262.

Van Rie, J., Jansens, S., Hoftey, H., Degheele, D. and Van Mellaert, H. (1989). Specificity of *Bacillus thuringiensis* - endotoxins. Importance of specific receptors on the brush border membrane of the midgut of target insects. *European Journal of Biochemistry* 186: 239-247.

Van der salm, T., Bosch, D., Honee, G., Feng, I., Munsterman, E., Bakker, P., Stiekema, W. J. and Visser, B. (1994). Insect resistance of transgenic plants that express modified Cry1A (b) and Cry1C genes: A resistance management strategy. *Plant Molecular Biology* 26: 51-59.

Walker-Simmons, M. and Ryan, C. A. (1977). Immunological identification of proteinase inhibitors I and II in isolated tomato leaf vacuoles. *Plant Physiology* 60: 61-63.

Wang, C. Z. and Qin, J. D. (1996). Effect of soybean trypsin inhibitor, gossypol and tannic acid on the midgut protease activities and growth of *Helicoverpa armigera* larvae. *Acta Entomologica Sinica.39*: 337-341.

Wang, C. Z., Xiang, X. F., Zhang, S. F. and Qin, J. D. (1995). Effect of soybean trypsin inhibitor on the growth and digestive physiology of *Helicoverpa armigera* larvae. *Acta Entomologica Sinica* 38: 272-277.

Watkins, W. M. and Morgan, W. T. J. (1952). Neutralization of the anti-H agglutinin in eel by simple sugars. *Nature* 169: 825-826.

Williams, S., Friedrich, L., Dincher, S., Carozzi, N., Kessmann, H., Ward, E. and Ryals, J. (1993). Chemical regulation of *Bacillus thuringiensis* δ-endotoxin expression in transgenic plants. *BioTechnology 7*: 194-200.

Wilson, A. G. L. (1982). Past and future *Heliothis* management in Australia. Proceedings of the International Workshop on *Heliothis* Management. (eds., W. Reed and V. Kumble). 15-20 November 1981, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru, A. P., India. pp. 343-354.

Wilson, F. D., Flint, H. M., Deaton, R. W., Fischhoff, D.A., Perlak, F. J., Armstrong, T. A., Fuchs, R. L., Berberich, S. A., Parks, N. J. and Stapp, B. R. (1992). Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. *Journal of Economic Entomology* 85: 1516-1521.

Wolfensberger, M. G. (1989). Neither barium nor calcium prevents the inhibition by *Bacillus thuringiensis* - endotoxin of sodium or potassium gradient dependent amino acid accumulation by tobacco hornworm midgut brush border membrane. *Archives of Insect Biochemistry and Biophysics 12*: 267-277.

Wunn, J., Kloti, A., Burkhardt, P.K., Biswas, G.C.G., Launis, K., Iglesias, V. A. and Potrykus, I. (1996). Transgenic indica rice breeding lines IR 58 expressing a synthetic CrylA(b) gene from *Bacillus thuringiensis* provides effective pest control. *BioTechnology* 14: 171-176.

Yeh, K. W., Liu, M. I., Tuan, S. J., Chen, Y. M., Liu, C. Y. and Kao, S. S. (1997). Sweetpotato (*Ipomoea batatus*) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against *Spodoptera litura*. *Plant Cell Reports* 16: 696-699.

Zhao, J.Z., Fan, X. L., Shi, X. P., Zhao, R.M. and Fan, Y.L. (1997). Gene pyramiding: an effective strategy of resistance management for *Helicoverpa armigera* and *Bacillus thuringiensis*. *Resistant Pest Management* 9: 19-21.

Zhao, R. M., Shi, X.P., Wang, J. H. and Fan, Y. L. (1996). Transgenic tobacco plants expressed both *Bt* and CpTI genes and their homozygotes. *Rice Biotechnology Quarterly* 25: 35-36.

VITA

The author of this thesis, Sonali Shukla, daughter of Shri Girish Kumar Shukla, was born on 30th May 1975 at Itarsi (M.P.). She has passed Higher School Certificate Examination in the year 1990, and higher secondary School Certificate Examination in the year 1992, in first division form Board of Secondary Education M.P., Bhopal. Then she joined B.Sc. (Agriculture), Jabalpur (JNKVV) in 1993. She completed the course with first division in year 1997. After graduation she joined in M.Sc. (Agriculture) in the Department of Entomology, College of Agriculture, Jabalpur (JNKVV) in 1998.