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Biological activity of soybean trypsin inhibitor and plant lectins against cotton bollworm/legume pod borer, *Helicoverpa armigera*

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Abstract The noctuid, *Helicoverpa armigera*, is the most important crop pest worldwide. We evaluated the biological activity of soybean trypsin inhibitor and plant lectins against this pest to identify toxin genes for deployment through transgenic plants. Of the seven plant lectins tested, chickpea and snowdrop lectins showed marked antibiotic effects in terms of insect survival and development. Larval survival was lower in artificial diet impregnated with soybean trypsin inhibitor (49%), and snowdrop (64%) and chickpea (65%) lectins compared to untreated control diet (90%). Pupal weight was adversely affected by chickpea lectin (272.6 mg) compared as to untreated control (335.4 mg). Lower pupation and/or adult emernence (<50%) was observed in diets impregnated with soybean trypsin inhibitor and chickpea, snowdrop and peanut lectins as compared to 90% pupation/adult emergence on untreated control diet. Soybean trypsin inhibitor, and lectins from snowdrop, peanut, and chickpea can be considered for deployment through transgenic plants for the management of *H. armigera*.

Key words: Soybean trypsin inhibitor, plant lectins, *Helicoverpa armigera*.

Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner), is one of the most devastating crop pests worldwide. It is a major pest of crops such as cotton, pigeonpea, chickpea, tomato, vegetables, and fruits. Due to indiscriminate use of insecticides to control this pest, it has developed a high level of resistance to conventional insecticides (Kranthi et al. 2002). Therefore, it is important to develop alternative methods of controlling this pest, including host plant resistance. However, the levels of resistance in the cultivated species are quite low (Sharma 2001), and there is an urgent need to explore the possibilities of using novel genes through genetically modified plants to increase the levels and diversify the bases of resistance to this difficult to control pest. Considerable progress has been made in developing transgenic plants with toxin genes from *Bacillus thuringiensis* (*Bt*) in different crops (Hilder and Boulter 1999; Sharma et al. 2000). However, there are distinct possibilities of development of resistance to *Bt* (Tabashnik 1994), and hence, the need to identify alternative genes such as protease inhibitors, secondary metabolites, and plant lectins for deployment through transgenic crops to control this pest.

Protease inhibitor proteins are produced in plant tissues and act as a defensive mechanism in response to insect attack (Ryan 1990). The first report on the use of a plant-derived insect control protein gene in transgenic plants came with the expression of cowpea trypsin inhibitor gene in tobacco (Hilder et al. 1987). Soybean trypsin inhibitor has been shown to reduce the growth of *H. armigera* (Johnston et al. 1993; Wang et al.1996). Another group of compounds, which is being exploited to impart resistance to insects in crop plants, is plant lectins. Lectins affect the survival and development of insect pests belonging to different orders (Shukle and Murdock 1983; Czapla and Lang 1990; Gatehouse et al. 1995; Powell et al. 1998; Bell et al. 1999). This paper reports the biological effects of soybean trypsin inhibitor and seven plant lectins on the growth and development of *H. armigera*, to identify candidate genes for deployment through transgenic plants for controlling this pest.

Materials and methods

The lectins and the soybean trypsin inhibitor used in the experiment were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Phosphate saline buffer, used to dissolve the lectins, was prepared by mixing 51.0 ml of A [0.2 M solution of mono-basic sodium phosphate (27.8 g in 1000 ml double distilled water)] and 49.0 ml of B [0.2 M solution of dibasic sodium phosphate (53.65 g of Na_2HPO_4 $7H_2O$ or 71.7 g of $Na_2HPO_4 12H_2O$ in 1000 ml double distilled water)] diluted to a total of 200 ml with distilled water.

Biological activity of plant lectins and soybean trypsin inhibitor impregnated into artificial diet

Plant lectins [Concanavalin A (*Canavalia ensiformis*), jacalin (*Artocarpus integrifolia*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), peanut (*Arachis hypogaea*), wheatgerm (*Triticum aestivum*), soybean (*Glycine max*) and snowdrop lectins (*Galanthus nivalis*)] and soybean trypsin inhibitor were tested at 0.1% concentration through impregnation into the artificial diet. Fifty mg of each lectin was dissolved in five ml of phosphate saline buffer (pH 6.8). Similarly, 50 mg of soybean trypsin inhibitor was dissolved in five ml of distilled water. Five ml of the dissolved lectins or the soybean trypsin inhibitor was added to forty-five ml of artificial diet (Armes et al. 1992) and stirred on a magnetic stirrer for mixing the ingredients. The control treatment consisted of 5 ml of distilled water or 5 ml buffer at pH 6.8 added to forty-five ml of artificial 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 laboratory table. The larvae were released on the artificial diet with a camelhair brush. Ten neonate larvae were released in each replication. There were five replications for each treatment in a completely randomized design. The vials were kept in the insect rearing laboratory at 27±2°C, 65±5% relative humidity, and 12 h photoperiod. Larval weights and mortality were recorded on fifth day after initiating the experiment. After data recording, ten larvae in each replication (one larva in each vial) were placed back into the artificial diet and observations were recorded on the date of pupation and adult emergence. Pupal weights were recorded one day after pupation. The remaining larvae were weighed on an electronic balance, and then killed in chloroform after 4 h of starvation and oven dried at 65°C for 72 h. Oven dry weights of the larvae were recorded on an electronic balance. The data were subjected to analysis of variance. The significance of differences between the treatment means was judged by F-test, while the treatment means were compared by using least significant difference at P=0.05.

Biological activity of plant lectins and soybean trypsin inhibitor through surface treatment of artificial diet

Seven plant lectins (Concanavalin A, jacalin, chickpea, lentil, peanut, wheatgerm, and snowdrop lectins) were bio-assayed against *H. armigera* by treating the surface of the artificial diet (5 ml) in the glass vials (2 cm diameter and 3.5 cm length) with 100 μl of the 1 or 2% solution of different lectins. The lectin solutions were prepared in phosphate buffer as described above. The solution was spread uniformly over the diet surface, and allowed to dry under the table fan in the laboratory for five hours. Five neonate larvae were released in each vial, and there were 50 larvae in each replication. There were three replications in a completely randomized design. Observations were recorded on weight of the larvae five days after initiating the experiment. One larva was placed back in each vial (to avoid cannibalism) to record observations on postembryonic development period. In another experiment, bio-efficacy of concanavalin A and C and trypsin inhibitor was studied at 1 and 2% level in the artificial diet against *H. armigera*. The other experimental details were similar to those described above. One neonate larva was released in each vial, and there were 50 larvae in each replication. There were three replications in a completely randomized design. Observations were recorded on larval mortality and larval weights at 5 and 10 days after initiating the experiment, pupation, adult emergence, and sex ratio. The data were subjected to analysis of variance. The significance of differences between the treatment means was judged by F-test, while the treatment means were compared by using least significant difference at P=0.05.

Results

Biological activity of plant lectins and soybean trypsin inhibitor impregnated into artificial diet

Larval survival varied from 49% in larvae reared on diet impregnated with SBTI to 90% in larvae reared on the control diet at 5 days after initiating the experiment (Table 1). Amongst the lectins tested, larval survival was significantly low in larvae reared on diet impregnated with snowdrop (64%), followed by chickpea (65%), and jacalin (78%). Concanavalin A, lentil, soybean, and peanut lectins did not affect the survival of *H. armigera* larvae when impregnated into artificial diet. Fresh and dry weights of the larvae were lower in control diet as compared to the larvae reared on diets containing SBTI and plant lectins. Pupal weights 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, as compared to 335.4 mg in larvae reared on the untreated control diet. There were no adverse effects of the plant lectins on pupal weight, except in larvae reared on diet with chickpea lectin. Percentage pupation (of the total larvae released) ranged from 40% in diet impregnated with SBTI to 90% in untreated control diet. (Figure 1) Amongst the lectins tested, low pupation was observed in larvae reared on diets impregnated with chickpea (50%), snowdrop (60%), and soybean lectins (70%). Percentage adult emergence (of the total larvae released) was lowest in larvae reared on diet containing snowdrop lectin (20%), followed by those reared on diets containing SBTI (30%), and chickpea (30%), peanut (38%), jacalin (48%), concanavalin A (58), and soybean (65%) lectins, as compared to 90% adult emergence on untreated

Table 1. Effect of plant lectins and soybean trypsin inhibitor (0.1%) impregnated into the artificial diet on larval survival and weight gain by *Helicoverpa armigera*.

Treatment	Larva survival (%) (5 DAI)	Larval fresh weight (mg/larva) (5 DAI)	Larval dry weight (mg/larva) (5 DAI)	Pupal weight (mg/pupa)
Untreated control	90 (81.00)	2.40	0.50	335.4
Buffer	86 (75.69)	1.20	0.20	336.7
SBTI	49 (44.33)	3.50	0.50	323.5
ConcanavalinA	90 (76.72)	4.20	0.50	322.2
Lentil lectin	90 (76.72)	4.00	0.60	323.6
Jacalin	78 (69.23)	4.50	0.60	325.1
Soybean lectin	86 (74.18)	6.70	0.70	341.8
Peanut lectin	84 (73.03)	4.60	0.60	349.5
Snowdrop lectin	64 (55.03)	5.40	0.70	359.2
Chickpea lectin	65 (61.98)	7.30	0.80	272.6
SE	±6.7 (5.76)	±0.8	±0.07	±8
LSD at 5%	18.52 (15.92)	2.21	0.19	22.11

Plant lectins and trypsin inhibitor mixed with the artificial diet on a weight/volume basis (ICRISAT, Patancheru, 2000).
Values in parentheses are Angular transformed values. SBTI=Soybean trypsin inhibitor. DAI=Days after initiating the experiment.

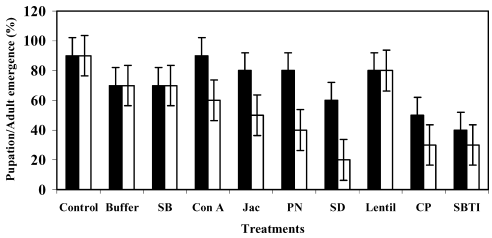

Figure 1. Effect of plant lectins and soybean trypsin inhibitor on *H. armigera*. Materials were impregnated in artificial diet and pupation (black bars) and adult emergence (white bars) of *H. armigera* (50 mg per 50 ml of diet) were estimated (ICRISAT, Patancheru, 2000). SB=Soybean lectin, Con A=Concanavalin A, Jac=Jacalin, PN=Peanut lectin, SD=Snowdrop lectin, CP=Chickpea lectin, and SBTI=Soybean trypsin inhibitor.

control diet (Figure 1).

Biological activity of plant lectins and SBTI through surface treatment of artificial diet

The weights of the larvae 5 days after initiating the experiment ranged from 14.88 mg in diet treated with 1% jacalin to 25.72 mg on diet with 1% chickpea lectin as compared to 24.14 mg in larvae reared on untreated control diet (Table 2). Larval weights were lower in larvae reared on artificial diets treated with concanavalin A (1%) and lentil lectin (1%). Lectins from peanut, chickpea, wheat germ, and snowdrop showed no adverse effect on the larval weights. Pupal weights varied from 203.98 mg on diet having 1% chickpea lectin to 294.5 mg in diet having lectin from wheat germ (1%), compared to 285.8 mg in untreated control diet. Total post-embryonic development was extended by over three days in artificial diet treated with lectins from chickpea (2%), lentil (1%), wheat germ (1 and 2%), and jacalin (2%).

In another experiment on comparison of the biological activity of concanavalin and soybean trypsin inhibitor, concanavalin A (2%), concanavalin C (1 and 2%), and trypsin inhibitor (2%) resulted in over 20% larval mortality as compared to 10% mortality in larvae reared on untreated control diet (Table 3). There was a significant reduction in larval weights at 10 days after initiating the experiment in diet having trypsin inhibitor (7.45 and 4.19 mg at 1 and 2%, respectively) as compared to the untreated control diet (16.96 mg). Concanavalin A and C did not show any marked effect on the larval weights (15.58 to 19.99 mg). There were no adverse effects of SBTI and concanavalin A and C on the pupal weights (296.95 to 319.36 mg) as compared to the larvae reared on the untreated control diet (310.07 mg). Post-embryonic development period was prolonged by over two days in larvae fed on artificial diets containing concanavalin C (2%) and trypsin inhibitor (2%). Percentage pupation ranged from 53.33% on diet treated with SBTI (2%) to 76.67% in larvae reared on untreated control diet, and the diet treated with phosphate buffer. Low pupation (<60%) was observed in larvae reared on diets treated with concanavalin A (2%), concanavalin C (2%), and SBTI (1%).

Discussion

In the diet impregnation assay, larval survival was significantly lower in larvae reared on diet impregnated with snowdrop lectin, chickpea lectin, and jacalin. Concanavalin A, lentil, soybean, and peanut lectins did not affect the survival of *H. armigera* larvae when impregnated into artificial diet. There were no adverse effects of the plant lectins on pupal weight, except in larvae reared on diet with chickpea lectin. The snowdrop lectin has previously been shown to be toxic to Homoptera (Rahbe et al. 1995; Powell et al. 1993), Lepidoptera (Fitches et al. 1997), and Coleoptera

Table 2. Effect of plant lectins on weight gain and post embryonic development of *Helicoverpa armiger*.

Treatment	Larval weight (mg/larva) (5 DAI)	Pupal weight (mg/pupa)	Post embryonic development period (days)
Untreated control	24.14	285.81	24.95
Buffer	23.17	264.47	25.47
ConcanavalinA (1%)	17.26	233.18	26.07
Peanut lectin (1%)	21.77	241.66	27.44
Jacalin (1%)	14.88	271.39	27.81
Jacalin (2%)	15.53	282.94	29.09
Chickpea lectin (1%)	25.72	203.98	26.53
Chickpea lectin (2%)	19.23	261.86	28.67
Snowdrop lectin (1%)	25.66	291.90	26.99
Lentil lectin (1%)	16.50	255.24	28.00
Lentil lectin (2%)	20.12	232.63	22.48
Wheat germ lectin (1%)	20.56	294.48	28.14
Wheat germ lectin (2%)	18.27	263.47	28.45
SE	±1.58	±28.98	±1.26
LSD at 5%	4.60	84.6	3.68

Lectins were applied on the surface of artificial diet. Larval and pupal weights and post-embryonic development period of neonate larvae of *Helicoverpa armigera* (surface treatment assay) were estimated (ICRISAT, Patancheru, 2001). A 100-μl of each test chemical was applied per 3.24 cm^2. DAI=Days after initiating the experiment.

Table 3. Biological activity of Concanavalin A and C and soybean trypsin inhibitor against *Helicoverpa armigera*.

Treatment	Mortality (%) (10 DAI)	Larval weight (mg) (5 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)	Pupation (%)
Control	10.00	16.96	310.07	14.79	8.50	76.67
Buffer	13.33	17.38	313.00	14.87	8.64	76.67
Concanavalin A (1%)	16.67	19.72	309.83	14.57	8.82	70.00
Concanavalin A (2%)	30.00	20.00	306.50	15.87	9.23	60.00
Concanavalin C (1%)	23.33	16.57	299.26	14.71	9.33	66.67
Concanavalin C (2%)	26.67	15.58	309.80	16.00	9.78	60.00
Soybean trypsin inhibitor (1%)	16.67	7.45	296.95	16.25	8.94	56.67
Soybean trypsin inhibitor (2%)	20.00	4.19	319.36	16.74	9.43	53.33
SE	±7.03	±1.51	±8.74	±0.3	±0.27	±10.01
LSD at 5%	21.32	4.59	26.51	0.91	0.82	80.36

Surface treatment assay was done as described (ICRISAT, Patancheru, 2001). A 100-μl of each test chemical was applied per 3.24 cm^2. DAI=Days after initiating the experiment.

(Gatehouse et al. 1995; Elden 2000). It resulted in feeding inhibition and reduction in the larval weight against the legume pod borer, *Maruca vitrata* (Machuka et al. 1999) and tomato moth, *Lacanobia oleracea* (Fitches et al. 1997). Fresh and dry weights of the larvae were lower in control diet as compared to the larvae reared on diets containing plant lectins in the diet impregnation assay. The increased weights of the larvae might be because of use of lectins as a source of additional protein. Similar effects of soybean lectin have been reported against *O. nubilalis* larvae (Czapla and Lang 1990) and sugarcane borer, *Diatraea saccharalis* (Setamou et al. 2002). In the surface treatment assay, larval weights were lower in *H. armigera* larvae reared on artificial diets treated with concanavalin A and lentil lectin, which might be because of greater concentration of these chemicals on the surface of the diet. Low pupation and/or adult emergence were observed in larvae reared on diets impregnated with SBTI, and lectins from chickpea, snowdrop, peanut, and jacalin. In the surface treatment assay, post-embryonic development period was extended by over three days in artificial diet treated with lectins from chickpea (2%), lentil (1%), wheat germ (1 and 2%), and jacalin (2%). The adverse effects of plant lectins were more apparent against larval pupation and adult emergence than on larval survival and growth.

Larvae of cotton budworm, *Heliothis virescens* fed on transformed cotton with the lectin gene have a reduced weight, but there was no effect on larval survival (Satyendra et al. 1998). Larval biomass of the tomato moth (*Lacanobia oleracea*) is reduced in an artificial diet containing snowdrop lectin, and on excised leaves of transgenic tomato (Fitches et al. 1997), which may result in lower fecundity of the female moths. Transgenic

maize expressing wheat agglutinin (WGA) has shown moderate activity against *O. nubilalis* and *Diabrotica* spp. (Maddock et al. 1991). Low pupation (<60%) was observed in larvae reared on diets treated with concanavalin A (2%), concanavalin C (2%), and SBTI (1%). Protease inhibitors of plants are involved in a number of functions, including the control of endogenous proteolytic enzymes (Richardson 1977). Soybean Kunitz type trypsin inhibitor (SBTI) and Soybean Bowman–Birk type trypsin-chymotrypsin inhibitor (SBBI) have been shown to reduce larval weights of *H. armigera* in artificial diet (Johnston et al. 1993). Soybean trypsin inhibitor also affects the growth and digestive physiology of *H. armigera* (Wang et al. 1996).

Transgenic tobacco plants expressing trypsin inhibitor gene resulted in increased mortality, reduced insect growth, and reduced plant damage by *H. virescens* (Hilder et al. 1987), *H. zea* (Hoffman et al. 1992), *Spodoptera littoralis* and *Manduca sexta* (Yeh et al. 1997; McManus et al. 1999), and *H. armigera* (Zhao et al. 1998; Charity et al. 1999). However, Nandi et al. (1999) reported that *H. armigera* larvae fed on transgenic tobacco expressing *SBTI* gene showed normal growth and development. Three soybean protease inhibitor genes (KTi_3, *C-II*, and *PI-IV*) when transformed into tobacco and potato showed variable expression among different plants (Marchetti et al. 2000). Transgenic tobacco plants expressing high levels of *SBTI* have shown greater resistance than the tobacco plants expressing cowpea trypsin inhibitors (*CpTI*) against *H. virescens*. The SBTI is also more effective than CpTI in reducing the proteolytic activity of gut extracts obtained from full-grown larvae of *H. armigera*. However, CpTI is considered to be more useful for genetic transformation, because unlike many serine protease inhibitors (SPIs), it is not deleterious to mammals (Puzstai et al. 1992). Jhang et al. (2002) observed that a sub-lethal dose of Bt strain HD1 supplemented with SBTI in artificial diet did not result in significant differences in larval mortality. Supplementing a non-lethal dose of *Bt* with SBTI led to a pronounced reduction of larval growth. Snowdrop lectin, chickpea lectin, jacalin, and SBTI produced adverse effects on the survival and development of *H. armigera*. These can be considered as candidate genes for use in genetic transformation of crops for minimizing the losses due to *H. armigera*. However, despite several reports on successful protection of plants against phytophagous insects through SBTI and plant lectins, defense strategies based on protease inhibitor and lectin expression in plants have not resulted in any commercial applications so far. This could be due to the insects' capacity to react to these genes, and the levels of expression of these chemicals in plants. However, trypsin inhibitors and plant lectins can be used in conjunction with *Bt* genes to increase the levels, and diversify the basis of resistance to *H. armigera* in transgenic plans.

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