Biological Activity of Lectins from Grain Legumes and Garlic against the Legume Pod Borer, *Helicoverpa armigera*

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Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner), is one of the most devastating crop pests worldwide (Sharma 2001). It has a wide host range, and feeds on more than 300 plant species. Due to indiscriminate use of insecticides, it has developed high levels 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 to *H. armigera* in the cultivated germplasm of several crops are low to moderate. Therefore, improving plant resistance to pests through genetic transformation has raised hopes of using plant resistance as an effective weapon for pest management (Sharma et al. 2004). This includes incorporation of novel genes such as crystal protein from *Bacillus thuringiensis* (Bt-Cry genes), enzyme inhibitors (such as protease and alpha amylase inhibitors), vegetative insecticidal proteins (VIPs), small RNA viruses (SRVs), and secondary plant metabolites (SPMs). While the activity of Bt-Cry proteins has been investigated extensively, there is very little information on the biological activity of other insecticidal genes that can be used to confer resistance to insects in transgenic plants (Hilder and Boulter 1999). Therefore, we evaluated the biological activity of plant lectins as candidate genes for conferring resistance to *H. armigera*.

Lectins are carbohydrate-binding proteins (or glycoproteins) of non-immune nature, and bind reversibly to specific mono- or oligo-saccharides (Goldstein et al. 1980, Van Damme et al. 1998). They play an important role in the plant's defense against insect pests, and have been found to be toxic to viruses, bacteria, fungi, insects and higher animals. This paper reports the biological effects of plant lectins from field bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), chickpea (*Cicer arietinum*), and garlic (*Allium sativum*) along with snowdrop (*Galanthus nivalis*) lectin on the growth and development of *H. armigera* so as to identify the candidate genes for deployment through transgenic plants to control this pest.

Lectins extracted from chickpea, pigeonpea, garlic (garlic lectin I = from garlic leaves; garlic lectin II = from transgenic tobacco) and field bean were bio-assayed along with snowdrop lectin against the neonate larvae of *H. armigera*. The lectins were bio-assayed against the neonate larvae of *H. armigera* by treating the surface of the artificial diet (Armes et al. 1992) in a glass vial (2 cm diameter and 3.5 cm height) with 100 ml of different lectins. Each glass vial contained 5 ml diet. The lectin solutions were prepared in phosphate buffer (pH 6.8, molarity 0.2 M). The buffer was prepared by mixing 51.0 ml of A [0.2 M solution of mono-basic sodium phosphate (27.8 g in 1000 ml)] 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)] diluted to a total of 200 ml with distilled water. Lectins dissolved in phosphate buffer were spread uniformly over the diet surface with a micropipette, and allowed to dry under the table fan in the laboratory for 4 h. One neonate larva was released in each vial and observations were recorded on weight of the larvae five days after initiating the experiment, and larval, pupal, and total development period. Each treatment was replicated three times in a completely randomized design. There were 10 larvae in each treatment. Observations on larval weights were recorded 5 days later, while pupal weights were recorded one day after pupation. Data were also recorded on adult emergence. The data were subjected to analysis of variance.

The weights of the larvae at 5 days after initiating the experiment ranged from 16.54 mg on the artificial diet with buffer to 26.90 mg in diet treated with field bean lectin as compared to 22.68 mg in the untreated control diet (Table 1). However, the differences in larval weights in diets with different lectins were not significant. The larval weights were also quite low in the diet treated with phosphate buffer only. This may be because of some effects of the buffer on the pH of artificial diet. However, no adverse effects of the buffer were observed on larval and pupal periods and the pupal weights. The weight of the pupae reared on diet containing garlic lectin II (from transgenic tobacco) was significantly lower (283.81 mg per larva) as compared to those fed on untreated control diet (325.00 mg per larva). None of the lectins tested showed any adverse effect on larval period. Pupal period of the insects reared on diet containing lectins from field bean, pigeonpea, chickpea and garlic, was significantly shorter than those reared on the untreated control diet.

The differences in percentage pupation and adult

Table 1. Bio-efficacy of lectins extracted from grain legumes and garlic on survival and development of legume pod borer, *Helicoverpa armigera* (ICRISAT Patancheru 2002).

Treatment	Dosage (mg per cm^2)	Larval weight (mg) (5 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Sex ratio (females:males)
Untreated control diet	–	22.68	325.00	14.101	15.633	76.67	46.67	1:1.18
Artificial diet with buffer	–	16.54	317.16	14.500	12.500	73.33	43.33	1:0.86
Field bean lectin	0.032	26.90	305.66	14.258	12.194	66.67	36.67	1:0.34
Pigeonpea lectin	0.032	22.02	312.00	14.222	11.917	60.00	33.33	1:0.56
Chickpea lectin (1.72 mg/ml)	0.032	22.65	306.20	14.333	11.711	80.00	43.33	1:1.56
Chickpea lectin (in 60% $(NH_4)_2SO_4$)	0.032	22.25	304.29	14.222	11.083	60.00	36.67	1:0.75
Chickpea lectin (6 mg/ml)	0.032	24.48	315.46	14.073	11.744	66.67	40.00	1:1.26
Garlic lectin I (from garlic)	0.032	20.95	297.09	14.458	12.056	56.67	33.33	1:1.05
Garlic lectin II (from transgenic tobacco)	0.032	22.83	283.81	14.535	12.622	60.00	33.33	1:1.14
Snowdrop lectin	0.032	24.12	308.57	14.151	12.322	70.00	40.00	1:1.44
Mean		22.54	307.52	14.29	12.38	67.00	38.67	–
SE		±2.311	±11.96	±0.318	±1.144	±6.55	±6.95	–
LSD at 5%		6.87	35.55	NS	3.40	NS	NS	–

DAI = Days after initiating the experiment. NS = Non-significant.

emergence were not significant. However, less than 60% pupation was recorded in diets treated with lectins from pigeonpea, chickpea in 60% ammonium sulphate solution, garlic, and garlic lectin extracted from transgenic plants as compared to 76.67% in untreated artificial diet. Adult emergence ranged from 33.33% in diets treated with pigeonpea and garlic lectin to 46.67% in untreated control diet. The sex ratio (males:females) was affected adversely in diets treated with lectins from field bean and pigeonpea.

Anti-insect properties of the plant lectins have earlier been reported against European corn borer, *Ostrinia nubilalis* (Czapla and Lang 1990). The snowdrop lectin (GNA) has previously been shown to be toxic to Homoptera (Rahbe et al. 1995; Powell et al. 1995, 1998), Lepidoptera (Fitches et al. 1997), and Coleoptera (Gatehouse et al. 1995; Elden 2000). Snowdrop lectin (2%) inhibited feeding and reduced the weight of spotted pod borer, *Maruca vitrata* larvae (Machuka et al. 1999) and tomato moth (*Lacanobia oleracea*) (Fitches et al. 1997). Such effects of GNA were not observed in the present studies, possibly because of low concentrations used in the present studies.

Lectins have been reported to affect the survival and development of insect pests (Janzen et al. 1976; Shukle and Murdock 1983; Czapla and Lang 1990; Habibi et al. 1993; Gatehouse et al. 1993, 1995; Powell et al. 1995; Law and Kfir 1997). They bind to the glycan receptors present on the surface lining of the insect gut (Pusztai and Bardocz 1996), and interfere with the formation and integrity of the peritrophic membrane of the midgut (Harper et al. 1998), but how that affects the digestive physiology is unknown. Larval weights were slightly greater in diets treated with GNA, chickpea lectin, and field bean lectin. Similar effects of soybean lectin have earlier been reported in case of *O. nubilalis* (Czapla and Lang 1990). Percentage pupation was low (<60%) in diets treated with pigeonpea lectin, chickpea lectin in 60% ammonium sulphate solution, and garlic lectin, while adult emergence was low in diets treated with pigeonpea and garlic lectin. The garlic lectin had an adverse effect of the larval and pupal weights of *H. armigera*, but not on the duration of larval and pupal development. The lectins from garlic and pigeonpea can possibly be deployed in transgenic plants in combination with Bt genes to increase the levels of plant resistance to *H. armigera*.

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