Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Effect of nitrogen fertigation and sowing time on the expression of Cry2Ab and on mortality of *Spodoptera litura* in Bollgard II cotton

Manpreet Kaur Saini* and A.K. Dhawan

Department of Entomology, Punjab Agricultural University, Ludhiana-141 004, India

*Corresponding Author E-mail: mksaini@pau.edu

Publication Info

Paper received:
20 September 2012

Revised received:
02 July 2013

Accepted:
31 July 2013

Abstract

Toxin expression of *Cry2Ab* was studied in plant parts of Bollgard II cotton genotype MRC 7031 sown under different treatments of nitrogen application and planting dates. The expression was quantified by using *Cry2Aa* ELISA kit. Mean per cent mortality of one-day-old, 3rd and 5th instar larvae of *Spodoptera litura* was observed on different plant parts of MRC 7031 and their respective non-Bt cotton genotypes. The study deduced that mean maximum expression (19.24, 20.93 and 20.71 µg g^{-1} in leaves, squares and bolls, respectively) of Cry2Ab was observed at higher nitrogen dose @ 300 kg ha^{-1} (N_3), while it was minimum (18.67, 20.44 and 20.14 µg g^{-1} in leaves, squares and bolls, respectively) at low nitrogen dose @ 150 kg ha^{-1} (N_1). Studies conducted for different planting dates showed mean maximum expression (18.98, 20.72 and 20.42 µg g^{-1} in leaves, squares and bolls, respectively) of *Cry2Ab* during late sown crop (15th May) as compared to early sown crop (15th April), the expression was 18.66, 20.32 and 20.06 µg g^{-1} in leaves, squares and bolls, respectively. Quantitative expression of *Cry2Ab* was found to vary among different plant parts, i.e more in squares followed by bolls and leaves. Regarding mortality of different instars of *S. litura*, it was significantly more at higher nitrogen doses and it ranged from 83.04 to 96.27, 53.38 to 61.87 and 16.87 to 22.58 % in case of *S. litura* one-day-old larvae, 3rd and 5th instar, respectively. While, non significant difference in mortality was observed during different sowing dates.

Key words

Bollgard II cotton, *Cry2Ab*, Nitrogen doses, Planting dates, *Spodoptera litura*

Introduction

Nitrogen is an essential macronutrient required by all plants to thrive. It is an important component of many structural, genetic and metabolic compounds in plant cells. Nitrogen is one of the factor that directly influence vegetative growth and dry matter production. It is also one of the basic components of chlorophyll, by which plants use sunlight energy to produce sugars during the process of photosynthesis. Increasing the levels of nitrogen during the vegetative stage can strengthen the roots, enabling plants to take in more water and nutrients. This allows a plant to grow more rapidly and produce large amounts of succulent, green foliage, which in turn can generate higher yield. Using too much nitrogen, however, can be as harmful to plants as too little. Being a constituent of proteins, purines, pyrimidines and many coenzymes, a deficiency of nitrogen interferes with protein synthesis and growth of plant (Bruns and Abel, 2003). Nitrogen also enhances the Cry protein concentration in the plant (Ian, 2006), which later on gives more and more protection to the crop from pest incidence. Thus, nitrogen management in Bt-cotton assumes greater importance to boost the productivity by protecting the crop from bollworm incidence. *Bt* cotton is considerably effective for lepidopteran pests and is beneficial in reducing insecticide sprays and thus preserving population of beneficial arthropods (Tabashnik *et al*., 2002). But the efficacy of *Bt* cotton against lepidopteran pests is not consistent over the growing season (Adamczyk and Sumerford, 2001), with plant age (Wan *et al.,* 2005), plant parts (Abel and Adamczyk, 2004), type of genes and gene insertion sites (Jackson *et al.,* 2004), environmental conditions (Mahon *et al.,* 2002). Bollgard II cotton has demonstrated significantly better control of bollworms, *Spodoptera* spp. and other lepidopteran pests than that observed with Bollgard cotton (Gore *et al.,* 2003). But variation in the efficacy of BG and BG II cotton as well as the involved

mechanisms need to be understood fully, so as to plan rational resistance management strategies to retard the rate of the development of resistance and to control target pests effectively by enhancing endotoxin expression through genetic or agronomic practices. For this reason, some well-validated bioassay techniques and enzyme-linked immunosorbent assays (ELISA) (Holt *et al*., 2002) have been established to quantify changes in the concentration of *Bt* toxin proteins. Keeping in view, the present study was conducted to find out the effect of nitrogen doses and planting dates on expression of *Cry2Ab* as well as on mortality of *Spodoptera litura* in Bollgard II cotton.

Materials and Methods

MRC 7031 Bollgard II (BG II) genotype along with their respective non-*Bt* genotype were sown with three different planting dates viz.15th April (D_1), 1st May (D_2) and 15th May (D_3), during the year 2006 and 2007 at Entomological Research Farm, PAU, Ludhiana. Different doses of nitrogen viz. recommended (150 kg ha^{-1}) (N_1), 1.5 times recommended (220 kg ha^{-1}) (N_2), 2.0 times recommended (300 kg ha^{-1}) (N_3) and control (without any dose) (N_0) were applied in separate plots. The expression of *Cry2Ab* was recorded in leaves, squares and bolls of Bollgard II cotton genotype sown under different treatments. Samples were collected at different time intervals viz. leaves: 60, 90, 120, 140 and 180 days after sowing (DAS); squares: 60, 90, 120 and 140 DAS and bolls : 90, 120, 140 and 180 DAS. Leaves were collected from 3rd terminal, squares from Ist internode which was 5-6 day old, bolls from 2nd internode which was 8-10 day old were collected and transported to the laboratory under cool and dark conditions. Estimation of *Cry2A* was done with ELISA kit of ENVIROLOGIX 500 (Riverside Industrial Parkway Port Land Marine, U.S.A) following manufactures protocol.

Estimation of *Cry2Ab* toxin: Toxin was estimated by using *Cry2A* ELISA Kit that contained its own negative control solutions, different calibrators, enzyme conjugate, alkaline phosphatase substrate and stop solution. For extraction of *Cry2Aa*, 20 mg tissue of leaf, square and boll was collected in the sample extractor, and the further procedure was followed as per the manufacturer's protocol given in the *Cry 2A* ELISA kit. Finally, the result was presented as microgram toxin per gram of tissue.

Laboratory bioassay: Laboratory bioassay with one-day-old, 3rd and 5th instar larvae of *Spodoptera litura* was conducted on different plant parts viz. leaves, squares and bolls. The plant samples were washed with running water and cleaned with muslin cloth. Then samples of MRC 7031 and their respective non-*Bt* genotype sown under different treatments were placed separately in the vials containing agar solution. Later on, the larvae were released separately on different plant parts. The plant parts were changed every day and mortality was recorded after every 24 hr interval till 7 day of release or till pupation in case of 5th instar larvae. Corrected mortality was calculated by Abbott's formula (Abbott, 1925).

Statistical analysis: Observations recorded on different parameters were analyzed by using Factorial Completely Randomized Design (fCRD).

Results and Discussion

Among different nitrogen doses viz. 150 kg ha^{-1} (N_1), 225 kg ha^{-1} (N_2), 300 kg ha^{-1} (N_3) and control, the expression of *Cry2Ab* was found to be significantly more at N_2 and N_3 doses in different plant parts (Table 1) as compared to N_1 dose. The mean expression of endotoxin at 300 kg ha^{-1} was 19.24 µg g^{-1} in leaves, 20.93 µg g^{-1} in squares and 20.71 µg g^{-1} in bolls. The similar trend was reported by Pettigrew and Adamczyk (2006) that application of additional nitrogen 27.22 kg ha^{-1} elevated the *Bt* endotoxin expression by 12 % relative to treatments that only received 18.14 kg ha^{-1} nitrogen. Increasing doses of nitrogen to plants further enhanced the endotoxin synthesis or mRNA which later on increase *Cry* protein concentration. Thus, *Cry* protein content increased with increase in fertilizer levels and decreased with decreasing levels (Xuli and Liguo, 2000; Kumar *et al*., 2011). Similar study was conducted by Ian, (2006) and Yang *et al*. (2005) and it was evident that increase in nitrogen affected the protein synthesis in *Bt* plants and thus improving the concentration of *Cry* protein. Among different sowing dates viz. 15th April, 1st May and 15th May, the expression of *Cry2Ab* in different plant parts indicated that it was significantly more in crops sown on 15th May than in 1st May and 15th April sown crop ($F_{2, 24}$ = 747.29, P < 0.0001; $F_{2, 24}$ =1619.53, P < 0.0001 and $F_{2, 24}$ =1029.04, P < 0.0001 in leaves, squares and bolls, respectively). *Bt* endotoxin level in cotton planted during early April was 14 % lower than planted in early May, presumably due to remobilization of the leaf nitrogen to support the larger developing boll load in the early April planted cotton (Pettigrew and Adamczyk, 2006). The interaction between planting dates and nitrogen doses indicated that maximum toxin expressed in 15th May sown crop at 225 and 300 kg of nitrogen ha^{-1}.

Plant samples viz. leaves, squares and bolls during different intervals were collected and fed to one-day-old larvae of *S. litura so* as to study the effect of nitrogen doses and planting dates. The perusal of data in Table 2 revealed that when the leaves, squares and bolls were fed to one-day-old larvae of *S. litura*, the mean mortality rate at higher nitrogen dose differed significantly than mortality at low nitrogen dose ($F_{3, 24}$= 104.69, P < 0.0001, $F_{3, 24}$= 23.27, P < 0.0001 and $F_{3, 24}$= 10.35, P < 0.0001 in leaves, squares and bolls, respecively), while the mortality was significantly low at N_1 and N_0. Similarly, during different sowing dates, the mortality of *S. litura one-day-old* larvae on different plant parts leaves ($F_{2, 24}$ = 0.14, P= 0.8701), squares ($F_{2, 24}$= 0.82, P= 0.4524) and bolls ($F_{2, 24}$= 1.24, P= 0.3073) showed statistically non significant difference. The interaction between planting dates and nitrogen doses also differed non-significantly (P= 0.9456, 0.9627, 0.9456 in leaves, squares and bolls, respectively).

Perusal of data (Table 3) revealed that the leaves, squares and bolls fed to 3rd instar larvae of *S. litura*, showed that

Table 1 : Effect of nitrogen doses and planting dates on expression of *Cry 2Ab* in different plant parts

Nitrogen dose (kg ha^{-1})	Variation in *Cry2Ab* (μg g^{-1}) content											
	Leaves				Squares				Bolls			
	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean
N_1(150)	18.51	18.65	18.85	18.67	20.24	20.45	20.63	20.44	19.97	20.13	20.33	20.14
N_2(225)	19.08	19.25	19.36	19.23	20.70	20.96	21.11	20.92	20.51	20.62	20.79	20.64
N_3(300)	19.09	19.21	19.41	19.24	20.72	20.94	21.14	20.93	20.44	20.79	20.91	20.71
N_0(Control)	17.97	18.12	18.28	18.12	19.60	19.81	19.98	19.80	19.33	19.50	19.66	19.50
Mean	18.66	18.81	18.98		20.32	20.54	20.72		20.06	20.32	20.42	

CD (p=0.05)

Plant parts	Date of sowing	Nitrogen doses	Date of sowing x Nitrogen doses
Leaves	0.01	0.02	0.03
Squares	0.02	0.02	0.04
Bolls	0.01	0.02	0.03

Table 2 : Effect of nitrogen doses and planting dates on mortality of *S. litura* (one-day-old larvae) on different plant parts

Nitrogen dose (kg ha^{-1})	Per cent mortality* of one-day-old larvae of *S. litura*											
	Leaves				Squares				Bolls			
	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean
N_1 (150)	72.51	72.08	72.43	72.34	92.26	92.55	93.68	92.83	85.68	87.25	88.42	87.12
N_2(225)	83.36	84.48	83.45	83.76	94.93	96.99	95.96	95.96	91.18	91.18	91.28	91.21
N_3(300)	82.27	83.20	83.65	83.04	95.75	95.86	97.19	96.27	89.61	91.67	93.33	91.54
N_0(Control)	70.79	69.91	71.12	70.61	88.46	88.77	89.08	88.77	84.90	84.42	85.90	85.07
Mean	77.23	77.42	77.66		92.85	93.54	93.98		87.84	88.63	89.73	

* Corrected mortality CD (p=0.05)

Plant parts	Date of sowing	Nitrogen doses	Date of sowing x Nitrogen doses
Leaves	NS	1.97	NS
Squares	NS	2.11	NS
Bolls	NS	2.87	NS

Table 3 : Effect of nitrogen doses and planting dates on mortality of *S. litura* (3rd instar larvae) on different plant parts

Nitrogen dose (kg ha^{-1})	Per cent mortality* of 3rd instar larvae of *S. litura*											
	Leaves				Squares				Bolls			
	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean
N_1(150)	46.51	46.80	47.63	46.98	54.62	55.03	55.41	55.02	51.83	52.29	53.03	52.38
N_2(225)	53.23	52.64	53.75	53.21	61.02	62.64	62.95	62.20	58.37	57.78	58.15	58.10
N_3(300)	51.90	53.53	54.71	53.38	62.43	61.34	61.85	61.87	57.57	59.20	59.71	58.83
N_0(Control)	43.61	43.61	43.91	43.71	51.46	52.62	53.28	52.45	49.01	49.47	50.07	49.52
Mean	48.81	49.15	50.00		57.38	57.91	58.37		54.20	54.69	55.24	

* Corrected mortality; CD (p=0.05)

Plant parts	Date of sowing	Nitrogen doses	Date of sowing x Nitrogen doses
Leaves	NS	3.38	NS
Squares	NS	3.86	NS
Bolls	NS	3.67	NS

Table 4 : Effect of nitrogen doses and planting dates on mortality of *S. litura* (5th instar larvae) on different plant parts

Nitrogen dose (kg ha^{-1})	Per cent mortality* of 5th instar larvae of *S. litura*											
	Leaves				Squares				Bolls			
	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean	15th April	1st May	15th May	Mean
N_1(150)	10.81	10.89	11.33	11.01	15.46	16.01	16.28	15.92	13.58	14.53	14.72	14.28
N_2(225)	15.85	17.01	16.58	16.48	20.18	19.60	20.15	19.98	20.30	23.17	22.63	22.03
N_3(300)	16.79	16.07	17.75	16.87	19.03	21.13	21.40	20.52	22.63	21.94	23.17	22.58
N_0(Control)	10.15	10.22	10.29	10.22	12.66	13.12	13.21	12.99	12.66	12.96	13.14	12.92
Mean	13.40	13.55	13.99		16.83	17.47	17.76		17.29	18.15	18.42	

* Corrected mortality; CD (p=0.05)

Plant parts	Date of sowing	Nitrogen doses	Date of sowing x Nitrogen doses
Leaves	NS	3.16	NS
Squares	NS	3.32	NS
Bolls	NS	3.87	NS

mean mortality per cent at N_2 and N_3 dose, differed significantly from mortality at N_1 dose ($F_{3,24}$= 17.08, P < 0.0001; $F_{3,24}$=14.25, P < 0.0001 and $F_{3,24}$ = 12.77, P < 0.0001 in leaves, squares and bolls, respectively). While during different sowing dates, the mortality of 3rd instar larvae of *S. litura* on different plant parts showed statistically non significant difference (P = 0.6946, 0.7508, 0.7962 in leaves, squares and bolls, respectively). The interaction between sowing time and fertilizer application differed non-significantly (P= 0.9957, 0.9983 and 0.9989 in leaves, squares and bolls, respectively).

When leaves, squares and bolls were collected from different treatments and fed to 5th instar larvae of *S. litura*, there was significant difference in mean mortality per cent at N_2 and N_3 doses (Table 4), than at N_1 dose ($F_{3,24}$= 10.54, P < 0.0001 in leaves; $F_{3,24}$= 9.79, P < 0.0002 in squares; $F_{3,24}$= 14.56, P < 0.0001 in bolls). Mortality of *S. litura* 5th instar larvae on different plant parts collected from different sowing dates showed statistically non significant difference (P= 0.8963, 0.7963, 0.7732 in leaves, squares and bolls, respectively). The interaction between planting dates and nitrogen doses also differed non-significantly (P= 0.9984, 0.9957 and 0.9944 in leaves, squares and bolls, respectively).

According to the present study, mortality among different instars showed that it was maximum in one-day-old larvae as compared to 3rd and 5th instar of *S. litura*. Similar study was conducted by Hallad *et al*. (2011), which showed that among 2nd, 3rd and 4th instar larvae of *Spodoptera litura*, the highest mortality was for 2nd instar larvae (80.30 %) at 80 DAS as compared to 3rd and 4th instar (72.60 and 64.20 respectively) recorded in Tulasi 4 BG-II.

Acknowledgments

My (MPK) heartiest appreciation goes to Prof. V.K. Dilawari, Dr. H.S. Rewal, Prof. M.S. Gill, Dr. Jai Singh for providing me feedback and valuable suggestions. I owe my sincere thanks and personal regards to Dr. N.S. Butter, Head, Department of Entomology for providing the necessary facilities.

References

Abbott, W.S.: A method of computing the effectiveness of insecticides. *J. Econ. Entomol.*, **18**, 265-267 (1925).

Abel, C.A. and J.J. Adamczyk: Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera:Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) on maize whorl profiles. *J. Econ. Entomol.*, **97**, 1737-1744 (2004).

Adamczk, J.J. and D.V. Sumerford: Potential factors impacting season-long expression of Cry1Ac in 13 commercial varieties of Bollgard cotton. *J. Insect Sci.*, **1**, 1-6 (2001).

Bruns, H.A. and C.A. Abel: Nitrogen fertility effects on Bt endotoxin and N concentrations of maize during early growth. *Agron. J.*, **95,** 207-211 (2003).

Gore, J., B.R. Leonard and R.H. Gable: Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. *J. Econ. Entomol.*, **96**, 699-705 (2003).

Hallad A., S.S. Udikeri, S.B. Patil, B.M. Khadi, D.P. Biradar, K. Basavana Goud and A.R.S. Bhat: Characterization of resistance of different cry toxins to early and late instar *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.). *Karnataka J. Agric. Sci.*, **24**, 300-302 (2011).

Holt, H.E., C.L. Mares and R. Akhurst: Determination of the Cry1 protein content of Bt transgenic cotton-a technical manual for laboratory use. CSIRO *Entomol. Tech. Report,* **92**, 1-13 (2002).

Ian, J.R.: Effect of genotype, edaphic, environmental conditions and agronomic practices on *Cry 1 Ac* protein expression in transgenic cotton. *J. Cotton Res.*, **10**, 252-262 (2006).

Jackson, R.E., J.R. Bradley Jr., J.W. Van Duyn and F. Gould: Comparitive production of *Helicoverpa zea* (Lepidoptera: Noctuidae) from transgenic cotton expressing either one or two *Bacillus thuringiensis* proteins with and without insecticide oversprays. *J. Econ. Entomol.*, **97**, 1719-1725(2004).

Kumar, R.L., B.V. Patil and N.G. Vijaykumar: Impact of irrigation and

fertilizer levels on *Cry 1 Ac* protein content in *Bt* Cotton. *Res. J. Agric. Sci.*, **2**, 33-35 (2011).

Mahon, R., J. Finnergan, K. Olsen and L. Lawrence : Environmental stress and the efficacy of *Bt* cotton. *Aust. Cottongrower*., **22**, 18-21 (2002).

Pettigrew, W. T. and J. J. Adamczyk Jr: Nitrogen fertility and planting date effects on lint yield and *Cry1Ac (Bt)* endotoxin production. *Agron. J.,* **98**, 691-697(2006).

Tabashnik, B.E., T.J. Dennehy, M.A. Sims, K. Larkin, G. P. Head, W.J. Moar and Y. Carriere: Control of resistant pink bollworm that produces *Bacillus thruingiensis* toxin *Cry2Ab*. *Appl. Environ. Microbiol.,* **68,** 3790-3794 (2002).

Wan, P., Y. Zhang, K. Wu and M. Huang: Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for Bt cotton in the Yangtze River Valley of China. *J. Econ. Entomol.*, **98**, 195-201(2005).

Xuli, H. and F. Liguo: Influence of fertilizer and environmental temperature on the resistance of Bt. transgenic cotton to cotton bollworm. *J. Anhuai Agric. Univ.*, **4**, 235-237 (2000).

Yang, Chang-qin., Li-hua Xu and De-Yin Yang: Effect of nitrogen fertilizer on Bt-protein contentin transgenic cotton and nitrogen metabolism mechanism. *Cotton Sci.*, **17**, 227-231 (2005).