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Changes in baseline susceptibility of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to Cry1Ac toxin from *Bacillus thuringiensis.*

Abstract

Changes in the geographical variability in *H. armigera* susceptibility levels to Cry1Ac toxin from *Bacillus thuringiensis*, was monitored through log dose probit assays conducted on populations collected from 7 cotton-growing districts of North India, 15 populations of Central India and 5 populations of South India. The LC_{50} values derived from 9745 larvae tested, ranged from 0.092 to 0.790 µg Cry1Ac/ml of diet with 4.42-fold, 8.59- fold and 3.18 –fold variability in susceptibility across the North, Central and South Indian strains of *H. armigera*. The IC_{50} values ranged from 0.010- 0.156 µg Cry1Ac/ml of diet with 19.5 fold variability across the country.

Introduction

Insecticidal genes belonging to the Cry1A group has been the most widely used for the generation of transgenic crops. Cry1Ac is a crystal protein derived from the soil bacterium *Bacillus thuringiensis* (Bt). It is toxic to lepidopteran insects including the cotton bollworm *Helicoverpa armigera*[1], which is one of the most important economic insect pests in many parts of the world including Asia, Australia and Africa. Bollgard, which expresses a single gene *cry1Ac* has been in cultivation in the USA for almost a decade and is considered as one of the best technological advances for cotton pest management. Bt cotton was introduced in India since 2002 and over five years its area has increased from 30,000 hectares to 37 lakh hectares in 2006-07 with no report of field control failures of *H. armigera*, so far However, the deployment of single genes for the expression of insecticidal toxin proteins in crop plants is expected to select for resistance in the target pests over a period of continuous exposure. The capacity of *H. armigera* to develop resistance to Cry1Ac has been demonstrated by laboratory selection in Australia[1], China[2] and India[3]. Although field control failures of bollworms have not yet been reported from any country including India, till date, availability of full grown *H. armigera* larvae on Bt cotton especially in Gujarat is gradually increasing over the years. However, populations of *H. armigera* collected from Bt cotton are not being used to monitor changes in baseline susceptibility. Populations are specifically collected from non- Bt cotton if available or on hosts other than cotton.

For resistance management programmes to be effective, monitoring, surveillance and early detection of resistance are important prerequisites. Regular monitoring for resistance development helps to detect the emergence of resistant phenotypes in order to initiate timely remedial measures[4]. Since the first studies wherein baseline monitoring for the bollworms was reported [5-7] our lab has developed a database on the changes in baseline susceptibility to Cry1Ac in *H. armigera* monitored each year from populations collected on cotton and on other crops. It is important that resistance development to the Cry1Ac in the target pest is monitored carefully so that it is not allowed to increase to levels that impair its efficacy. The current study aims to understand the changes in geographical variability of the baseline susceptibility in the cotton bollworm, *H. armigera* to the Cry1Ac toxin in North, Central and South India five years after introduction of Bollgard for commercial cultivation.

Materials and Methods

Laboratory strains of *H. armigera* were established from larvae collected in cotton fields during the cropping season of 2006-2007 from major cotton growing regions of North India. Field strains of the cotton bollworm *H. armigera* were collected during October 2006, on non- Bt cotton fields from 7 districts of three states in North India (Sirsa and Hissar of Haryana, Bhatinda, Fatehabad, Abohar, Dabhwali of Punjab and Sriganganagar of Rajasthan). The percentage emergence of moths from field collected larval populations in the F_0 generation ranged from 46.67 % in Sriganganagar to 90.00 % in Hissar. This ensured the availability of a total of 4925 larvae representing the seven locations, for Cry1Ac bioassays. Part of the data pertaining to this study formed a mid term report of the year 2006-07. *H. armigera* larvae were also collected from fields of Central India with special emphasis on the cotton growing districts of Gujarat. Field collected populations from Nagpur, Amravati, Yavatmal and Parbhani represented Central Indian population from Maharashtra while populations from Junagadh, Surat, Vadodara and Baruch represented Gujarat. Larvae were also collected from cotton fields of Dharwad (Karnataka), Mehbubnagar, Warangal, Guntur and Nalgonda (Andhra Pradesh). A total of 1220 larvae represented South India while 3600 larvae represented Central India in Cry1Ac bioassays Larvae were reared on a chickpea based semi-synthetic diet[8] individually in 7.5 ml cells of 12 well 'ICN-Linbro' tissue culture plates until pupation. Moths were kept in glass jars and fed on 10 % honey solution. A layer of muslin cloth was placed on the inner surface of the jar for oviposition. One-day old larvae were tested at the rate of one larva per well at a total of twenty-four larvae per concentration on semi-synthetic diet incorporating different concentrations of the toxin. MVP II containing 19.6% Cry1Ac was tested at 5 concentrations in diet-incorporated bioassays. Cry1Ac in MVP II is 99% identical to the active toxin region of Cry1Ac expressed in Bt cotton. Mortality was recorded daily until the sixth day. The assays were performed in the laboratory at conditions of 27 $\pm 1^0$C and 70% relative humidity. Median Lethal Concentration (LC_{50}) presented in Table 1 was derived from log dose probit calculations[9]. IC_{50} values have been presented in Table 2.

Results and Discussion

The geographical variability in *H. armigera* susceptibility levels to Cry1Ac was not very different as compared to the variability observed in 1999 before introduction of Bollgard. The LC_{50} values ranged from 0.094 μg Cry1Ac/ml of diet (Sriganganagar) to 0.416 μg Cry1Ac/ml of diet (Bhatinda) for populations of *H. armigera* from North India. The variability in susceptibility across the strains was 4.42 fold. The fiducial limits (FL) at 90% probability, and the χ^2 values of the probit assay data indicated that the variability in response of the different *H. armigera* populations to Cry 1Ac was reducing over the years.

The LC_{50} values of populations from Central India (other than Gujarat) ranged from 0.092 μg Cry1Ac/ml of diet with populations from Buldana to 0.640 μg Cry1Ac/ml of diet (Yavatmal). The variability in susceptibility across populations was 6.96 fold. The LC_{50}s of populations of Gujarat ranged from 0.520 μg Cry1Ac/ml of diet with populations of *H. armigera* from Junagadh to 0.790 μg Cry1Ac/ml of diet with *H. armigera* populations from Surat. The variability in susceptibility across populations from across Gujarat was just 1.52 fold.

The highest LC_{50} of South Indian population tested was 0.576 μg Cry1Ac/ml of diet with populations from Warangal and the lowest LC_{50} value was 0.181 μg Cry1Ac/ml of diet with *H. armigera* populations from Dharwad. The variability of the LC_{50} of *H. armigera* populations from South India was a mere 3.18 fold.

IC_{50} expressed as μg Cry1Ac/ ml of diet represents the concentration of the toxin that prevents 50% of the treated larvae from reaching the third instar (Kranthi *et. al*., 2005). IC_{50} values ranged from 0.013 (Sriganganagar) -0.058 μg Cry1Ac/ ml of diet (Bhatinda) for North India, from 0.010 (Buldana)- 0.195 μg Cry1Ac/ ml of diet (Surat) for Central India and from 0.021 (Mahbubnagar) to 0.147 μg Cry1Ac/ ml of diet (Warangal) for South India. The variability in IC_{50} values ranged from 4.46 fold, 19.5 fold and 7- fold with *H. armigera* populations of North, Central and South India. The variability in IC_{50} values was 19.5 fold across the country.

The changes in LC_{50} values in 2006 as compared to 2005 did not indicate signs of resistance development in any of the *H. armigera* populations examined. For example, the LC_{50} values that were between 0.110 and 0.374 μg Cry1Ac/ml during 2005 for the populations made from North India appeared to have changed marginally to a range of 0.094 to 0.416 μg Cry1Ac/ml in field populations collected during 2006. The lowest LC_{50} value recorded in both years was from Sriganganagar of Rajasthan. The highest LC_{50} value for North India was recorded from Bhantinda in 2005 and in 2006. Variability in the LC_{50} values was 3.4 fold for North Indian populations of *H. armigera* in the year 2005-06 and 8.59 fold in 2006-07.

With respect to Central Indian populations of *H. armigera* the highest LC_{50} value of 0.719 μg of Cry1Ac/ ml of diet was observed with populations from Vadodara and the same was true this year, except that populations of Surat also demonstrated a high LC_{50}. The lowest LC_{50} values in Central India was observed with populations of *H. armigera* collected from Buldana in 2005-06 (0.107 μg Cry1Ac/ml of diet) and in 2006-07 (0.092 μg Cry1Ac/ml). The published LC_{50} data of *H. armigera* baseline susceptibility to Cry1Ac in India, prior to the introduction of Bt cotton in the country, were within the range of 0.01 to 0.71 μg Cry1Ac/ ml of diet. Therefore changes in the current data does not indicate significant disturbance in the baseline susceptibility of *H. armigera* to Cry1Ac in North, South or Central India including Gujarat.

Resistance monitoring data showed a progressive decline in the overall variability between *H. armigera* populations with reference to their susceptibility to Cry1Ac over the five-year period after the introduction of Bollgard I. The baseline LC_{50} data obtained in 1999 showed 63-fold variability between *H. armigera* populations that reduced to 27 fold in 2002, 12 fold in 2004, 8.9 fold variability in 2005 and 8.28 fold in 2006. Availability of populations of *H. armigera* on cotton whether Bt or non-Bt has significantly declined over the years. Normally, two peak larval populations are observed on rain-fed cotton in central India. Today, populations of *H. armigera* in Central India are restricted to a single peak on cotton occurring in August- September. Populations of *H. armigera* were tested from about 27 locations this year of which 2 populations (7.4 %) demonstrated LC_{50}s lower than the composite LC_{50} of 0.10 μg Cry1Ac/ml of diet. One population of *H. armigera* from North India, 1 population from Central India demonstrated LC_{50} values less than the composite LC_{50} value of 0.10 μg Cry1Ac/ ml of diet, this year.

The variations in Cry1Ac LC_{50} between populations, prior to the introduction of Bollgard I in 1998-99 were 63 fold in the country (Kranthi *et. al*., 2001). Today it stands at a maximum of 8.28 fold after five years of exposure to GM cotton. These changes do not indicate the onset of resistance but are significant as they indicate the beginning of a progressive decline in the proportion of *H. armigera* populations susceptible to Cry1Ac. CICR currently maintains the database on the changes in geographic variability in susceptibility of *H. armigera* populations, to Cry1Ac over years, since 1999.

Acknowledgements

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Table 1: Variability in median lethal (LC_{50}) baseline susceptibility of *H. armigera* to Cry1Ac in field populations collected from twenty-seven locations of North, Central and South India

Location	n	LC_{50}	95% (F.L)	LC_{90}	95% (F.L)	Slope ± SE	X^2	df
NORTH INDIA								
Bhatinda	240	0.416	0.147- 1.407	5.559	1.584-145.711	1.139 ± 0.122	6.723	3
Abohar	648	0.214	0.137- 0.334	3.812	1.981-9.871	1.025 ± 0.071	5.521	3
Sirsa	648	0.276	0.203- 0.352	5.135	3.258-9.134	0.998 ± 0.071	2.849	3
Fatehabad	845	0.131	0.072- 0.237	1.396	0.658-4.956	1.247 ± 0.072	15.928	3
Hisar	648	0.139	0.084-0.226	1.973	1.002-5.540	1.111 ± 0.076	7.509	3
Sriganganagar	648	0.094	0.051- 0.167	1.135	0.542-3.867	1.186 ± 0.081	6.028	3
Dabwali	1248	0.245	0.209- 0.287	4.162	3.195-.632	1.041 ± 0.051	1.674	3
CENTRAL INDIA								
Buldhana	240	0.092	0.014- 0.430	1.092	0.275- 294.839	1.192 ± 0.133	11.429	3
Nagpur	240	0.120	0.045- 0.304	1.257	0.450-13.422	1.255 ± 0.135	5.583	3
Wardha	240	0.100	0.019- 0.472	0.895	0.245-175.477	1.345 ± 0.147	11.970	3
Jalgaon	240	0.285	0.091- 0.986	4.175	1.147-139.508	1.099 ± 0.119	7.159	3
Parbhani	240	0.223	0.086- 0.589	3.162	1.038-38.584	1.114 ± 0.120	5.264	3
Amravati	240	0.325	0.090- 1.427	4.743	1.165-386.313	1.101 ± 0.119	8.715	3
Akola	240	0.399	0.114- 1.806	5.073	1.279-409.750	1.160 ± 0.124	9.071	3
Aurangabad	240	0.426	0.134- 1.698	4.774	1.315-226.533	1.221 ± 0.130	8.498	3
Yeotmal	240	0.640	0.332- 1.299	3.431	1.605-17.615	1.758 ± 0.201	4.497	3
Chindwara	240	0.264	0.078- 0.978	4.115	1.076-185.124	1.075 ± 0.117	7.577	3
Junagarh	240	0.520	0.254- 1.160	5.872	2.251-39.261	1.217 ± 0.132	3.798	3
Surendranagar	240	0.559	0.259- 1.318	5.329	2.023-42.636	1.309 ± 0.143	4.599	3
Bharuch	240	0.564	0.200- 1.851	4.062	1.374-105.261	1.494 ± 0.165	8.209	3
Vadodara	240	0.760	0.534- 1.106	6.322	3.730-13.486	1.393 ± 0.154	2.013	3
Surat	240	0.790	0.347- 2.086	5.868	2.190-65.542	1.442 ± 0.160	5.780	3
SOUTH INDIA								
Nalgonda	240	0.352	0.097- 1.280	4.548	1.187-231.694	1.119 ± 0.120	8.082	3
Warangal	240	0.576	0.255- 1.424	3.595	1.447-33.089	1.611 ± 0.178	6.054	3
Guntur	240	0.557	0.277- 1.205	5.102	2.076-31.000	1.332 ± 0.145	3.975	3
Mahbubnagar	240	0.201	0.075- 0.538	2.795	0.911-36.548	1.121 ± 0.121	5.507	3
Dharwad	240	0.181	0.074- 0.438	2.273	0.815-20.224	1.167 ± 0.125	4.875	3

Table 2: Variability in Inhibition concentration (IC_{50}) baseline susceptibility of *H. armigera* to Cry1Ac in field populations collected from twenty-seven locations of North, Central and South India

Location	n	IC_{50}	95% (F.L.)	IC_{90}	95% (F.L.)	Slope ± SE (M)	X^2	df
NORTH INDIA								
Bhatinda	240	0.058	0.011 - 0.193	0.698	0.207 - 40.049	1.190 ± 0.141	8.468	3
Abohar	648	0.053	0.012 - 0.151	0.626	0.204 - 16.7	1.193 ± 0.144	7.033	3
Sirsa	648	0.019	0.011 - 0.03	0.2	0.121 - 0.437	1.260 ± 0.18	1.501	3
Fatehabad	845	0.032	0.008 - 0.074	0.437	0.164 - 4.711	1.123 ± 0.148	4.605	3
Hisar	648	0.016	0.008 - 0.024	0.163	0.098 - 0.359	1.261 ± 0.195	1.285	3
Sriganganagar	648	0.013	0.006 - 0.024	0.282	0.156 - 0.702	0.965 ± 0.149	1.0825	3
Dabwali	1248	0.014	0.006 - 0.023	0.187	0.109 - 0.433	1.126 ± 0.176	1.292	3
CENTRAL INDIA								
Buldhana	240	0.010	0.004 - 0.018	0.137	0.08 - 0.319	1.142 ± 0.193	0.934	3
Nagpur	240	0.013	0.006 - 0.021	0.169	0.099 - 0.39	1.133 ± 0.181	1.555	3
Wardha	240	0.017	0.008 - 0.027	0.219	0.129 - 0.499	1.143 ± 0.171	1.672	3
Jalgaon	240	0.021	0.01 - 0.034	0.356	0.201 - 0.849	1.034 ± 0.147	1.492	3
Parbhani	240	0.023	0.012 - 0.037	0.371	0.211 - 0.865	1.062 ± 0.147	1.017	3
Amravati	240	0.083	0.027 - 0.227	0.872	0.299 - 13.739	1.257 ± 0.141	11.998	3
Akola	240	0.142	0.055 - 0.358	1.474	0.532 - 14.604	1.262 ± 0.134	5.715	3
Aurangabad	240	0.046	0.012 - 0.115	0.543	0.194 - 7.762	1.192 ± 0.147	5.671	3
Yeotmal	240	0.156	0.044 - 0.579	1.291	0.396 - 51.442	1.398 ± 0.147	9.897	3
Chhindwara	240	0.026	0.015 - 0.041	0.361	0.21 - 0.815	1.122 ± 0.152	0.959	3
Junagarh	240	0.062	0.009 - 0.238	0.758	0.208 - 102.654	1.182 ± 0.139	9.7899	3
Surendranagar	240	0.071	0.016 - 0.232	0.738	0.226 - 32.551	1.259 ± 0.144	8.596	3
Bharuch	240	0.090	0.015 - 0.424	0.897	0.237 - 235.430	1.282 ± 0.142	11.849	3
Vadodara	240	0.132	0.053 - 0.313	1.599	0.589 - 13.362	1.185 ± 0.128	4.863	3
Surat	240	0.195	0.073 - 0.510	2.932	0.957 - 36.783	1.088 0.119	5.181	3
SOUTH INDIA								
Nalgonda	240	0.043	0.008 - 0.123	0.578	0.185 - 17.473	1.136 ± 0.142	6.795	3
Warangal	240	0.147	0.052 - 0.400	1.742	0.580 - 24.136	1.193 ± 0.128	6.201	3
Guntur	240	0.142	0.042 - 0.476	1.267	0.400 - 37.078	1.348 ± 0.143	8.840	3
Mahbubnagar	240	0.021	0.007 - 0.04	0.195	0.092 - 0.995	1.310 ± 0.19	3.097	3
Dharwad	240	0.031	0.018 - 0.046	0.355	0.21 - 0.775	1.203 ± 0.159	2.829	3