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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*, were determined through log dose probit assays conducted on populations collected from 39 cotton-growing districts across India. The LC_{50} values ranged from 0.081 to 0.719 µg Cry1Ac/ml of diet with 8.9-fold variability in susceptibility across the strains. The IC_{50} range indicated 21-fold variability with the values ranging from 0.008 to 0.166 µg/ml. The probit analysis data represent the toxicity of Cry 1Ac to *H. armigera* four years subsequent to the introduction of Bollgard cotton in India.

Introduction

Cry1 Ac 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. 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].

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]. Thus, it is important that resistance development to the Cry 1Ac in the target pest is monitored carefully so that it is not allowed to increase to levels that impair its efficacy. Baseline susceptibility of *H. armigera* to Cry1Ac in India is known[5-7] and its changes are being monitored each year since the introduction of Bollgard. 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 India, four 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 2005-2006 from major cotton growing regions India. Field strains of the cotton bollworm *H. armigera* were collected during October-December 2005, on cotton fields from 19 districts of central India (Nagpur, Hingoli, Aurangabad, Parbhani, Wardha, Chindwara, Jalgaon, Warora, Amravati, Nanded, Akola, Yavatmal, Beed, Buldana, Bharuch, Vadodara, Surat, Surendranagar and Amreli), 9 districts of North India (Hanumangarh, Sirsa, Fatehabad, Sriganganagar, Abohar, Mansa, Dabhwali, Varanasi and Bhatinda) and 11 districts of South India (Warangal, Khammam, Karimnagar, Guntur, Adilabad, West Godavari, Nizamabad, Prakasham, Mancherial, Ongole and Dharwad). The strains were established on semi-synthetic diet. 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 per well at a total of twenty to twenty four larvae per concentration on semi-synthetic diet incorporating different concentrations of the toxin. MVP II containing 19.6% Cry 1Ac was tested at 5 concentrations in diet-incorporated bioassays. Cry 1Ac in MVP II is 99% identical to the active toxin region of Cry 1Ac expressed in Bt cotton. Mortality was recorded daily until the sixth day. Weights of surviving larvae were recorded on the final day of observation. The assays were performed in the laboratory at conditions of 27 $\pm 1^0$C and 70% relative humidity. Median Lethal Concentrations (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 Cry 1Ac was not very different as compared to the variability observed in 1999 before introduction of Bollgard. The LC_{50} values ranged from 0.081 to 0.719 μg Cry 1Ac/ml of diet. The range of LC_{50} was 0.110 (Sriganganagar) to 0.374 (Bhatinda) in north India, 0.093 to 0.719 in central India and 0.081 (West Godavari) to 0.609 (Guntur) μg/ml of diet in south India. The variability in susceptibility across the strains was 8.9 fold. The most susceptible LC_{50} value of 0.093 μg/ml was observed in populations collected from Beed district in Maharashtra and the highest value of 0.719 μg/ml from Vadodara in Gujarat, Central India. The IC_{50} range indicated 21-fold variability in *H. armigera* response to Cry1Ac. The populations collected from Beed and Buldana in central India exhibited the lowest value of 0.008 and 0.009 μg/ml, respectively, whereas the highest IC_{50} value of 0.166 μg/ml was observed in samples collected from Surat, followed by 0.142 for populations from Vadodara in Central India The fiducial limits (FL) at 95% 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 changes in LC_{50} values in 2005 as compared to the earlier years did not indicate signs of resistance development in any of the *H. armigera* populations examined. For example, the LC_{50} values that were between 0.02 and 0.54 during 2002, appeared to have increased marginally to a range of 0.06 to 0.73 in field populations collected during 2004 and 0.081 to 0.72 in 2005, respectively. The increase in IC_{50} values was also marginal. The values increased from an initial 0.003 to 0.043 observed during 2002 to 2-3 fold higher values in 2004 and 3-4 fold higher values (0.008 to 0.166) in 2005. The published LC_{50} data of *H. armigera* baseline susceptibility to Cry 1Ac in India, prior to the introduction of Bt cotton in the country, were within the range of 0.01 to 0.71 ug Cry 1Ac/ ml of diet. Therefore the marginal increase observed in the current data does not indicate significant disturbance in the baseline susceptibility of *H. armigera* to Cry 1Ac, because of the extensive overlap of the fiducial limits with the values reported in the baseline data. It must be mentioned here that countries like Australia, USA and China have been cultivating Bt cotton much before its cultivation in India. None of these countries have reported field failures of Bt cotton as a result of *H. armigera* till date but recent reports from Asia have again shown that *H. armigera* can develop resistance on transgenic cotton [10,11].

Resistance monitoring data showed a progressive decline in the overall variability between *H. armigera* populations with reference to their susceptibility to Cry 1Ac over the four-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 and 8.9 fold variability in 2005. Percentage of populations showing LC_{50} values less than the composite LC_{50} value of 0.10 ug Cry 1Ac/ ml of diet was 37% in 2002, 26% in 2004 and 5 % in 2005. Similarly, IC_{50} data showed that 59% of the *H. armigera* population showed IC_{50} values of less than 0.01 ug Cry 1Ac/ ml of diet in 1999. The same reduced to 22 % in 2003, 9 % on 2004 and 7.7 % in 2005. Between 2003 and 2004 the reduction in variability in *H. armigera* populations susceptibility to Cry 1Ac was 13 % while between 2004-05 the reduction in variability was only 1.3 %. 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 Cry 1Ac.

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 thirty -nine districts of India during 2005-06.

North India	**n**	**LC_{50}**	**95% FL**	**LC_{90}**	**95% FL**	**Slope ± SE**	**X^2**	**DF**
Sriganganagar	240	0.110	(0.076 - 0.159)	1.037	(0.630 - 2.08)	1.320 ± 0.14	2.270	3
Varanasi	240	0.126	(0.086 - 0.182)	1.27	(0.760 - 2.60)	1.270 ± 0.13	2.850	3
Hanumangarh	240	0.172	(0.036 - 0.823)	2.06	(0.515 - 308.3)	1.180 ±0.120	11.06	3
Bhatinda	240	0.374	(0.130 - 1.190)	2.91	(0.970 - 64.23)	1.43 ±0.15	8.21	3
Sirsa	240	0.222	(0.080 - 0.601)	2.779	(0.918 - 35.40)	1.160 ±0.120	5.760	3
Fatehbad	240	0.229	(0.085 - 0.638)	2.613	(0.866 - 35.50)	1.210 ±0.130	6.180	3
Abohar	240	0.269	(0.070 - 1.090)	2.86	(0.790 - 148.6)	1.24 ±0.13	9.52	3
Mansa	240	0.282	(0.104 - 0.820)	3.05	(0.820 - 47.18)	1.24 ±0.13	6.57	3
Dabwali	240	0.168	(0.119 - 0.237)	1.239	(0.780 - 2.360)	1.478 ±0.160	2.240	3
Central India								
Nagpur	240	0.130	(0.088 - 0.190)	1.4	(0.830 - 2.910)	1.240 ±0.13	2.390	3
Wardha	240	0.135	(0.067 - 0.266)	1.34	(0.587 - 6.177)	1.280 ±0.13	3.530	3
Hingoli	240	0.150	(0.078 - 0.281)	1.470	(0.677 - 5.840)	1.290 ±0.140	3.080	3
Chhindwara	240	0.178	(0.121 - 0.260)	1.94	(1.130 - 4.07)	1.240 ±0.13	2.450	3
Jalgaon	240	0.189	(0.094 - 0.379)	1.92	(0.830 - 9.12)	1.270 ±0.130	3.570	3
Parbhani	240	0.190	(0.050 - 0.740)	1.52	(0.460 - 67.03)	1.420 ±0.150	10.29	3
Warora	240	0.231	(0.110 - 0.480)	1.615	(0.710 - 8.11)	1.520 ±0.160	4.460	3
Amravati	240	0.236	(0.079 - 0.747)	2.49	(0.777 - 51.74)	1.250 ±0.130	7.530	3
Nanded	240	0.245	(0.094 - 0.669)	2.16	(0.760 - 25.77)	1.360 ±0.140	6.620	3
Aurangabad	240	0.322	(0.125 - 0.897)	2.26	(0.830 - 28.75)	1.51 ±0.16	7.27	3
Akola	240	0.602	(0.269 - 1.488)	4.517	(1.740 - 40.75)	1.46 ±0.16	5.5	3
Yeotmal	240	0.661	(0.253 - 2.050)	4.88	(1.680 - 93.62)	1.47 ±0.16	7.37	3
Beed	240	0.093	(0.063 - 0.135)	0.983	(0.587 - 2.063)	1.250 ±0.14	2.400	3
Buldhana	240	0.107	(0.073 - 0.155)	1.085	(0.653 - 2.210)	1.270 ±0.13	1.390	3
Vadodara	240	0.719	(0.430 - 1.220)	2.81	(1.560 - 9.210)	2.16 ±0.27	3.37	3
Surendranagar	240	0.469	(0.159 - 1.618)	3.35	(1.110 - 96.22)	1.5 ±0.16	8.86	3
Bharuch	240	0.512	(0.160 - 2.080)	4.02	(1.21) - 189.3)	1.43 ±0.15	9.68	3
Amreli	240	0.332	(0.111 - 1.120)	3.07	(0.95 - 77.11)	1.33 ±0.14	8.19	3
Surat	240	0.627	(0.418 - 0.950)	3.08	(1.84 - 6.99)	1.85 ±0.21	1.75	3
South India								
Guntur	240	0.609	(0.155 - 3.85)	5.43	(1.38 - 1450)	1.34 ±0.15	11.71	3
Adilabad	240	0.559	(0.303 - 1.06)	3.06	(1.48 - 12.92)	1.73 ±0.19	3.92	3
Warangal	240	0.575	(0.295 - 1.17)	2.95	(1.39 - 14.93)	1.8 ±0.2	4.67	3
W.Godavari	240	0.081	(0.054 - 0.119)	0.861	(0.516 - 1.777)	1.250 ±0.14	1.770	3
Nizamabad	240	0.252	(0.08 - 0.788)	2.68	(0.840 - 51.88)	1.250 ±0.130	7.320	3
Khammam	240	0.258	(0.138 - 0.49)	1.84	(0.870 - 7.260)	1.503 ±0.16	3.55	3
Karimnagar	240	0.199	(0.062 - 0.664)	1.88	(0.586 - 48.38)	1.310 ±0.140	8.450	3
Prakasam	240	0.218	(0.045 - 1.209)	2.100	(0.532 - 458.9)	1.3 ±0.140	12.58	3
Mancherial	240	0.119	(0.082 - 0.172)	1.11	(0.676 - 2.22)	1.320 ±0.14	1.520	3
Ongole	240	0.125	(0.055 - 0.283)	1.048	(0.427 - 7.088)	1.390 ±0.15	5.021	3
Dharwad	240	0.155	(0.106 - 0.225)	1.560	(0.933 - 3.190)	1.280 ±0.130	2.090	3

LC_{50} is expressed in µg/g diet; n = the number of test insects; S.E = Standard Error; FL = Fiducial Limits.

Table 2. Variability in median inhibitory (IC_{50}) baseline susceptibility of *H. armigera* to Cry1Ac in field populations collected from thirty-nine districts of India during 2005-06.

North India	n	IC_{50}	FL	IC_{90}	FL	Slope SE	X^2	Df
Sriganganagar	240	0.010	(0.005 - 0.017)	0.122	(0.073 - 0.279)	1.196 ±0.200	0.530	3
Varanasi	240	0.013	(0.007 - 0.020)	0.107	(0.067 - 0.229)	1.404 ±0.230	0.180	3
Hanumangarh	240	0.018	(0.011 - 0.027)	0.139	(0.088 - 0.287)	1.443 ±0.216	0.396	3
Bhatinda	240	0.075	(0.050 - 0.109)	0.786	(0.471 - 1.630)	1.256 ±0.142	1.993	3
Sirsa	240	0.030	(0.018 - 0.044)	0.288	(0.176 - 0.604)	1.298 ±0.170	2.370	3
Fatehbad	240	0.033	(0.021 - 0.049)	0.322	(0.196 - 0.267)	1.300 ±0.168	2.850	3
Abohar	240	0.067	(0.043 - 0.100)	0.845	(0.495 - 1.813)	1.167 ±0.134	0.816	3
Mansa	240	0.063	(0.042 - 0.0920	0.617	(0.373 - 1.268)	1.294 ±0.149	2.402	3
Central India								
Nagpur	240	0.015	(0.009 - 0.022)	0.092	(0.059 - 0.185)	1.627 ±0.260	0.108	3
Wardha	240	0.016	(0.010 - 0.022)	0.092	(0.060 - 0.183)	1.677 ±0.267	0.063	3
Hingoli	240	0.017	(0.010 - 0.024)	0.104	(0.067 - 0.210)	1.618 ±0.250	0.457	3
Chhindwara	240	0.019	(0.012 - 0.027)	0.121	(0.078 - 0.241)	1.586 ±0.237	0.119	3
Jalgaon	240	0.020	(0.014 - 0.032)	0.172	(0.108 - 0.325)	1.430 ±0.203	0.593	3
Parbhani	240	0.023	(0.013 - 0.035)	0.248	(0.150 - 0.529)	1.241 ±0.170	0.338	3
Warora	240	0.040	(0.010 - 0.101)	0.470	(0.168 - 7.340)	1.197 ±0.150	5.790	3
Amravati	240	0.042	(0.026 - 0.063)	0.500	(0.295 - 1.077)	1.189 ±0.147	2.808	3
Nanded	240	0.047	(0.030 - 0.070)	0.517	(0.309 - 1.089)	1.234 ±0.149	2.134	3
Aurangabad	240	0.067	(0.033 - 0.128)	0.670	(0.306 - 2.871)	1.128 ±0.147	3.192	3
Akola	240	0.097	(0.045 - 0.204)	0.639	(0.282 - 3.697)	1.566 ±0.175	4.806	3
Yeotmal	240	0.134	(0.092 - 0.019)	1.235	(0.753 - 2.467)	1.327 ±0.142	1.915	3
Beed	240	0.008	(0.002 - 0.014)	0.090	(0.057 - 0.223)	1.180 ±0.220	0.380	3
Buldhana	240	0.009	(0.004 - 0.016)	0.114	(0.068 - 0.263)	1.171 ±0.200	0.620	3
Vadodara	240	0.142	(0.099 - 0.203)	1.230	(0.755 - 2.430)	1.366 ±0.150	2.895	3
Surendranagar	240	0.078	(0.054 - 0.112)	0.689	(0.423 - 1.378)	1.357 ±0.152	2.877	3
Bharuch	240	0.085	(0.059 - 0.120)	0.682	(0.424 - 1.335)	1.413 ±0.157	2.503	3
Amreli	240	0.072	(0.048 - 0.105)	0.773	(0.461 - 1.164)	1.234 ±0.141	2.342	3
Surat	240	0.166	(0.080 - 0.165)	1.002	(0.617 - 1.978)	1.367 ±0.148	1.956	3
South India								
Guntur	240	0.108	(0.074 - 0.156)	1.046	(0.634 - 2.110	1.299 ±0.141	0.950	3
Adilabad	240	0.085	(0.042 - 0.166)	0.581	(0.270 - 2.691	1.534 ±0.172	3.996	3
Warangal	240	0.090	(0.042 - 0.190)	0.625	(0.274 - 3.616	1.525 ±0.170	4.705	3
W.Godavari	240	0.009	(0.004 - 0.015)	0.082	(0.050 - 0.184	1.330 ±0.250	0.500	3
Nizamabad	240	0.053	(0.035 - 0.078)	0.556	(0.334 - 1.159	1.256 ±0.149	2.560	3
Khammam	240	0.057	(0.035 - 0.086)	0.850	(0.485 - 1.196	1.089 ±0.129	1.070	3
Karimnagar	240	0.025	(0.015 - 0.039)	0.294	(0.175 - 0.634	1.206 ±0.162	0.147	3
Prakasam	240	0.027	(0.016 - 0.024)	0.336	(0.199 - 0.733	1.174 ±0.156	1.229	3
Mancherial	240	0.011	(0.005 - 0.018)	0.110	(0.067 - 0.240	1.297 ±0.220	1.190	3
Ongole	240	0.013	(0.007 - 0.019)	0.101	(0.063 - 0.214	1.424 ±0.240	0.270	3
Dharwad	240	0.017	(0.011 - 0.024)	0.093	(0.061 - 0.185	1.749 ±0.278	2.440	3

IC_{50} is expressed in µg/g diet; n = the number of test insects; S.E = Standard Error; FL = Fiducial Limits.