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Resistance to 'CrylAc δ-endotoxin of *Bacillus thuringiensis*' in a laboratory selected strain of *Helicoverpa armigera* (Hubner)

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The cotton bollworm, *Helicoverpa armigera* (Hubner) was selected for resistance to CrylAc in the laboratory. In the first 4–5 regimens of selection, there was no apparent change in the susceptibility of *H. armigera* to CrylAc. However, initial indications of resistance were clear after the 6th selection regimen, and by the end of 10th generation resistance increased 76-folds as reflected by the LC_{50} values. Similarly, resistance factors with respect to the EC_{50} increased to 34-fold by end of the 10th generation. The slope, which was relatively steep at 1.8 in the first generation, declined to 0.68 by the end of the 11th generation, indicating an increase in the number of resistant heterozygous individuals in the final population. A laboratory strain that was maintained without any selection pressure for 10 generations did not exhibit any change in susceptibility to CrylAc toxin.

Bt transgenic cotton expressing CrylAc is on the anvil of commercial release in India. The main target of the transgenic technology is the cotton bollworm *Helicoverpa armigera* (Hubner), which has been causing crop losses in India, estimated at US$ 290–350 million annually[1]. *H. armigera* has reportedly developed resistance to almost all groups of insecticides that have so far been used for its management[2], thus causing difficulties in field control. Resistance to *Bt* toxins expressed in plants is likely to develop in herbivorous insects under the right conditions[3]. Once in regular field use, insect resistance to CrylAc as well, is eventually expected to develop in lepidopteran insects, as has been the case with most toxins including insecticides. Also, the use of a single gene may lead to rapid development of resistance in species, which are more susceptible or even moderately susceptible to the toxin, thus reducing the impact of the technology as an effective lepidopteran insect management tool. Resistance development in insects against *Bt* toxins has been reported from field populations as well as laboratory-selected insects. Resistance to *Bt* toxins, as high as 1640-fold over a susceptible strain was found in a localized population of the diamond back moth, *Plutella xylostella* (L.) from Hawaii[3]. Laboratory selection programmes have generated resistance in Indian meal moth, *Plodia interpunctella* (Hub.)[4], Colorado potato beetle[5] and up to 10,000-fold in the cotton bollworm *Heliothis virescens* (Fab.)[6–8]. The main purpose of this study was to examine the potential of *H. armigera* to develop resistance to CrylAc under intense selection pressure in the laboratory.

The CrylAc proteins were produced according to Albert *et al.*[9], from *Escherichia coli* strains containing hyper-expressing recombinant plasmid vectors pKK223-3. The toxin was quantified on SDS-PAGE densitometry and diluted as six to ten concentrations (ranging from 10 to 20,000-fold) in distilled water. Forty per cent of the protein extracted from the recombinant *E. coli* cultures was found to comprise CrylAc toxin. Different concentrations of the toxin solutions were mixed thoroughly into a semisynthetic diet[10] pre-cooled to 55°C, at a rate of 2.4 ml of the toxin solution per 24 ml diet, and each concentration dispensed into a single 12-well 'ICN-Linbro' insect culture tray. A composite laboratory culture of *H. armigera* was initiated from pupae obtained from various parts of India from field-collected larvae. One-day-old larvae were released at the rate of one per well at a total of twenty-four larvae per concentration in three replicates on the diet incorporated with toxins. Larvae were transferred into toxin incorporated fresh diet trays, once in two days. Mortality was recorded daily till the sixth day, after which weights of the surviving larvae were recorded. The surviving larvae were then transferred on to fresh diet sans the toxin. All assays were replicated two to three times and pooled data was subjected to analysis. The assays were performed in the laboratory at conditions of 27 ± 1°C and 70% relative humidity. Median Lethal Concentrations (LC_{50}) pre-

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sented in Table 1, were derived from log dose probit calculations performed on POLO-PC software[11]. EC_{50} values representing the effective concentration that prevent 50% of individuals in the treated population from reaching half the average weight of control larvae, were also derived from POLO-PC software and are presented in Table 2. Resistance factors (RF) were calculated as LC_{50} of the selected strain/LC_{50} of the F1 strain.

During the course of the studies it was found that selection with an LC_{90} concentration often resulted in less number of progeny which was also difficult to maintain due to a reduced fecundity and hatching percentage. Moreover the numbers of insects available for continuing the selection pressure diminished progressively after each generation, presumably due to a fitness deficit in the surviving progeny. Hence, after the third selection regimen, the resulting progeny were selected only with an incremental increase in the dose of the selection concentration instead of the LC_{90} as was initially planned. Moreover, in order to maintain a reasonable number of test insects so as to sustain the selection experiments, three to four parallel colonies were established simultaneously from a relatively large number of pupae (200–300) and were considered as replicates. Each of such colonies was selected independently till the 6th generation after which all the survivors were pooled together to prevent the colonies from a probable collapse. However, by the end of the 11th generation there were not enough viable adults from the resulting progeny so as to enable continued selection.

Resistance factors were relatively low for the first four regimens of selection pressure. The first perceptible indications of resistance were clear only in the 6th generation when the fiducial limits of the F6 generation did not overlap with those of the F1 generation. Also, the LC_{50} and EC_{50} of the F6 strain increased by resistance factors of 6 and 8 fold, respectively, compared to the initial F1 generation. In the seventh generation, bioassays could not be carried out due to a reduction in the number of larvae available. Resistance increased rapidly to an LC_{50} related factor of 76-fold by end of the 10th generation and was 56-fold at the 11th generation. Similarly, the resistance factors with respect to the EC_{50} were 34 and 13 fold at the 10th and 11th generation respectively. Resistance was more clearly indicated in the LC_{90} data. The slope, which was relatively steep at 1.8 in the first generation, declined to 0.68 by the end of the 11th generation, indicating an increase in the number of resistant heterozygous individuals in the final population. A laboratory strain that was maintained without any selection pressure for 10 generations did not exhibit any change in susceptible response to the CrylAc toxin.

The LC_{50} toxicity values of CrylAc on *H. armigera* which were at 0.14 to 0.18 µg/ml of diet, indicate lesser susceptibility compared to the published LC_{50} of 0.01 to 0.03 µg/ml of *H. virescens*[8]. Further, *Bt* transgenic cotton crops which express CrylAc were found to cause 100% and 75–90% mortality in susceptible *H. virescens* and *Helicoverpa zea* (Boddie) respectively, in the US[12]. However, the same levels of expression, caused far less than 90% mortality of *H. armigera* and *Helicoverpa punctigera* (Wallengren) in Australia[13] suggesting that *Helicoverpa* species appear to be innately tolerant to the *Bt* toxins when compared to the *Heliothis* species.

The results clearly indicate that *H. armigera* is capable of developing resistance to CrylAc in 7–8 generations compared to *H. virescens* which was reported to resist any change in susceptibility for the first 12 selection episodes[8]. A number of laboratory and field populations of several different species of insects were selected for resistance with CrylAc[3,14]. Prominently, a laboratory strain of *H. virescens* was found to develop resistance levels of more than 500 and 10,000-fold to CrylAc after 19 and 30 progressive selection episodes respectively[8]. Our results with *H. armigera* indicate that the rate of resistance development is faster compared to *H. virescens*. This could probably be due to a higher

Table 1. Dose mortality response of *H. armigera* to CrylAc

Selection dose (µg/ml on previous generation)	LC_{50} (µg/ml)	(95% FL)	LC_{90} (µg/ml)	Slope ± SE	RF
F1		0.185*	(0.065 – 0.356)	0.95	1.80 ± 0.21	
F2	1.0	0.143*	(0.084 – 0.336)	1.143	1.42 ± 0.33	
F3	1.0	0.186*	(0.095 – 0.746)	2.655	1.11 ± 0.30	
F4	2.5	0.534*	(0.207 – 2.124)	7.469	1.12 ± 0.14	3
F5	5.0	0.325*	(0.143 – 4.020)	22.90	0.69 ± 0.22	2
F6	5.0	0.980	(0.413 – 10.25)	15.75	1.06 ± 0.28	6
F7	2.5					
F8	5.0	0.808	(0.340 – 8.515)	17.67	0.95 ± 0.25	5
F9	10.0	2.327*	(0.656 – 414.6)	54.42	0.93 ± 0.30	15
F10	10.0	11.50*	(2.07 – 18218)	907.4	0.67 ± 0.22	76
F11	10.0	8.544*	(1.79 – 3389.6)	624.7	0.68 ± 0.21	56

*Heterogeneity χ^2 significant at 5% level of significance.

Table 2. Dose response in growth inhibition of the CrylAc surviving *H. armigera* larvae

	Selection dose (µg/ml on previous generation)	EC_{50} (µg/ml)	(95% FL)	EC_{90} (µg/ml)	Slope ± SE	RF
F1		0.0136	(0.009 – 0.0193)	0.084	1.62 ± 0.26	
F2	1.0	0.0139*	(0.006 – 0.032)	0.057	2.09 ± 0.29	
F3	1.0	0.0117*	(0.005 – 0.0239)	0.043	2.27 ± 0.31	
F4	2.5	0.019*	(0.009 – 0.064)	0.130	1.54 ± 0.26	
F5	5.0	0.039	(0.028 – 0.0663)	0.176	1.97 ± 0.37	3
F6	5.0	0.110*	(0.051 – 1.133)	1.418	1.15 ± 0.32	8
F7	2.5					
F8	5.0	0.100*	(0.055 – 0.634)	0.523	1.78 ± 0.51	8
F9	10.0	0.103	(0.051 – 0.763)	1.106	1.24 ± 0.33	8
F10	10.0	0.452*	(0.115 – 333.55)	10.84	0.93 ± 0.38	34
F11	10.0	0.178*	(0.070 – 6.970	1.735	1.29 ± 0.42	13

*Heterogeneity χ^2 significant at 5% level of significance.

initial frequency of resistance alleles in field populations of *H. armigera* than those in *H. virescens*.

Interestingly, resistance to CrylAc in field populations of any of the lepidopteran insect pests, is yet to be detected in any part of the world, despite the fact that *Bt* transgenic cotton was being cultivated on a large-scale in the US, China and Australia over the past three years. This may have been made possible due to the implementation of *Bt* resistance management programmes right through inception of field use of the transgenic technology. But, after the introduction and large-scale cultivation of *Bt* transgenic cotton, it is reasonably certain that at some point of time *H. armigera* will respond to the intense selection pressure through a decline in its susceptibility to CrylAc, the gene used frequently against it. However, it would still be a matter of speculation if the rate of resistance development through laboratory selection could be related to what might happen under field conditions wherein a continuous gene-flow occurs in the form of dispersing and immigrant susceptible individual moths. Additionally, in farming systems such as in India where several crops are grown adjacent to each other, in vast mosaic patterns, the selection pressure may also be diluted due to the non-transgenic crops serving as hosts to *H. armigera*, thereby acting as reservoirs of susceptible refuges. Moreover, cotton crop is a prime host of *H. armigera* only for two to three months, which may cover just two generations, while for rest of the year the insect would survive on other non-transgenic crops. But, with the introduction of other *Bt* transgenic host crops such as tomato, maize, chickpea and pigeonpea, the resistance situation may aggravate due to a continuous selection pressure round the year. This would also pose a threat to the use of *Bt* sprays (most strains being used contain CrylAc as the major protein), which form an important component of Integrated Pest Management (IPM) programmes world over. Conversely, selection pressure due to *Bt* sprays also may render *H. armigera* less susceptible to the *Bt* transgenic crops.

CrylAc is highly toxic to the other two bollworms of cotton, namely spotted bollworm *Earias vittella* (Fab.)[15] and the pink bollworm *Pectinophora gossypiella* (Saund.)[16], but only marginally toxic to the tobacco caterpillar *Spodoptera litura* (Fab.) (Kranthi *et al.*, unpublished data). It is also important to prevent the re-emergence of the CrylAc tolerant *S. litura* as a major pest, especially in the wake of reduced pyrethroid usage on *Bt* cotton transgenic crops. One of the resistant management strategies is to use a combination of more than one gene such that together, the combination represents a high dose to a wide range of insect pests, while contributing to the delay in development of resistance[17]. For example, CrylF is toxic to *S. litura* (data not shown here) and can be used in conjunction with CrylAc, which was demonstrated as a synergistic combination against *H. armigera*[18]. Additionally, in order to effectively reduce the total insecticide use on cotton, it would be a good idea to transform cotton genotypes that are resistant to sucking pests, with Cry toxins, so that the plants would resist a wider range of pest complex.

Bt cotton transgenic crops, which are the products of intense scientific research involving high costs and efforts, indeed represent the state-of-the-art in pest management technology. Apart from the likelihood of reduction in insecticide use on transgenic cotton by at least 50–90%, it is also expected to ensure favourable ecological, economic and sociological returns, in contrast to the harmful effects due to the use of conventional insecticides. It is in the best interests of the farming community, that the benefits of such a technology be conserved and extended for the longest possible time. Since development of resistance is a evolutionary eventuality, it is imperative that studies be initiated to understand the basic nature of the phenomenon to enable combat the problem more effectively.

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Inflated pahoehoe lavas from the Sangamner area of the western Deccan Volcanic Province

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Notwithstanding the recent advances in petrology, geochemistry and determining the age of the Deccan Traps, there is no unanimity regarding the mechanism of their emplacement. Some of the basic questions regarding the source of the Deccan lavas, their actual eruption, and their post-eruptive behaviour have not been answered. In this paper, we describe the structure, physical characters and emplacement of some lava flows in the Sangamner area of the Deccan Volcanic Province (DVP). Our observations suggest that inflation (thickening by endogenous growth) was an important mechanism in the emplacement of flows in this area; and probably also in other parts of the DVP.

THE Deccan Volcanic Province (DVP) is one of the largest continental flood basalt provinces in the world. Considerable attention has been focused on this province in the last 10–15 years particularly because of its age, which corresponds closely with the Cretaceous–Tertiary boundary. Voluminous data regarding petrology, geochemistry, palaeomagnetism and age have emerged in the past two decades[1,2], but the physical volcanology of the lavas remains only sparsely recorded[3–6]. There is therefore considerable scope for physical volcanological studies that focus on the flow structures and thereby on the quantification of variables such as viscosity, temperature, volumetric flow rate, etc. This will help in the understanding of how the DVP lava flows and other flood basalt lavas were emplaced.

Studies in recent years on active lava flows in Hawaii[7], as well as those on the Columbia River Basalts[8,9] have shed considerable light on the emplacement of flood basalt lavas. These observations indicate that thick sheets of lava (about 4 m in Hawaii, 10–50 m in the Columbia Basalts) build up by the inflation and coalescence of thin (10–50 cm) pahoehoe lobes. Flows formed by this mechanism display certain diagnostic characters that are pointers to their mode of emplacement. Subsequently, such flows have been reported from the Mull area of Scotland, belonging to the North Atlantic Tertiary Province[10].

In this paper we discuss the structure, physical characters and emplacement of some lava flows in the area around Sangamner. Our observations[11] suggest that the mechanism of inflation played an important role in the emplacement of these lavas. Although compound pahoehoe flows have been recorded from several parts of the Deccan Traps[5,6], the mechanism of inflation has not been demonstrated before. This paper gives a detailed documentation of field evidences of inflation in the DVP, barring passing comments on their possible existence[2,12].

The area of investigation is bounded by the latitudes 19°25'N and 19°45'N and the longitudes 74°00'E and 74°15'E (Figure 1), and is close to the proposed eruptive centre for the Deccan basalts around the Igatpuri–Nasik region[13,14]. The basaltic flows exposed in this area belong to the lower and middle divisions of the Thakurwadi Formation of the Kalsubai Subgroup[1]. The

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