Synergistic effect of Cry1Ac and Cry1F δ-endotoxons of *Bacillus thuringiensis* on cotton bollworm, *Helicoverpa armigera*

Insecticidal δ-endotoxins of *Bacillus thuringiensis* (Bt) have acquired great significance in recent years because of their specificity to target insects, toxicity at very low concentration and environment friendly nature[1]. Genes coding for Bt δ-endotoxins have been deployed in a wide range of transgenic crop plants with considerable success[2]. One of the major concerns in field level application of Bt transgenic plants is development of resistance in insects towards δ-endotoxins upon continuous selection pressure[3]. Various strategies have been suggested to prevent or delay the resistance development among which gene pyramiding/mixture is an important measure[4]. A combination of Bt genes coding for δ-endotoxins which differ in their mode of action, receptor binding and sequence homology needs to be worked out in relation to each target insect. Recently we have reported the toxicity of eleven lepidoptera specific δ-endotoxins of Bt towards *Helocoverpa armigera*, an important polyphagous pest on cotton, chickpea, pigeonpea, tomato, sunflower,

Table 1. Toxicity of different combinations of Cry1Ac/Cry1F toxin mixtures to *H. armigera*

Ratio (Cry1Ac : Cry1F)	Observed EC^{*}_{50}	Slope ± SE	Expected EC^{**}_{50}	Expected/observed
1 : 0	8.0	0.86 ± 0.01	–	–
0 : 1	870.0	0.49 ± 0.03	–	–
2 : 1	0.5	1.15 ± 0.04	11.9	23.8
1 : 1	0.6	0.89 ± 0.02	15.8	26.3
1 : 2	1.9	1.15 ± 0.04	23.6	12.3

EC^{*}_{50}: Concentration of Cry toxins causing 50% larval growth reduction.

EC^{**}_{50}: Calculated by the formula used by Tabashnik[4].

sorghum, etc.[5]. Here we report synergism between two Bt δ-endotoxins in relation to their toxicity to *H. armigera*.

The genes (*cry1Ac, cry1Ab, cry1F* and *cry2Aa*) coding for Bt δ-endotoxins were overexpressed in *E. coli* strain JM103 using the expression vectors (from Donald Dean, Ohio State University, USA). The protoxins were purified and solubilized as described by Lee *et al.*[6]. The solubilized protoxin was digested with trypsin in a trypsin : protoxin ratio of 1 : 25 (by mass) for 2 h at 37°C. Activated toxins were dialysed against 50 mM sodium carbonate buffer, pH 9.5. The purity of the protoxin and activated toxin was examined on 10% SDS-PAGE[7]. The toxins at different concentrations were spread on semi-artificial diet in 24-well Costar plates and one larva (I instar) per well was released[5]. Larval mortality and growth inhibition were recorded after 6 days. The data were subjected to probit analysis[8]. Synergism between the toxins was calculated according to the equation of Tabashnik[9].

$$LD_{50(m)} = \left[\frac{r_a}{LD_{50(a)}} + \frac{r_b}{LD_{50(b)}} \right]^{-1},$$

where $LD_{50(m)}$ represents the expected LD_{50} of the mixture, $LD_{50(a)}$ and $LD_{50(b)}$ represent the LD_{50} for toxin a and b respectively, and r_a and r_b represent the relative proportion of toxins a and b in the mixture respectively.

We have previously observed that four toxins, viz. Cry1Ac, Cry1Aa, Cry1Ab and Cry2Aa were highly effective against *H. armigera* larvae and toxins such as Cry1F caused only growth inhibition[5]. Three combinations of toxins (Cry1Ac + Cry1Ab, Cry1Ac + Cry2Aa and Cry1Ac + Cry1F) were tested for their efficacy in the present study. No significant alteration in the toxicity was observed when the combinations of Cry1Ac + Cry1Ab and Cry1Ac + Cry2Aa were used (data not shown). On the other hand, Cry1Ac and Cry1F exhibited an interesting interactive effect (Table 1). Cry1Ac toxin is about 100 times more toxic than Cry1F toxin towards *H. armigera*. Mixture of Cry1Ac and Cry1F toxin (1 : 1) showed a synergistic effect in that the EC_{50} of Cry1Ac toxin was lowered 13 times due to the presence of Cry1F. The observed toxicity of the Cry1Ac + Cry1F mixture was about 26 times higher than the expected toxicity, strongly suggesting a synergism. Although we have no definitive explanation for the synergistic effect of the Cry1Ac and Cry1F mixture, two possible mechanisms can be speculated. The mechanism of Bt δ-endotoxin action involves binding of the toxins to the receptors, insertion into the membrane and pore formation[1]. It has been suggested that the toxin might oligomerize before or after binding to the receptors[10]. It is possible that a hetero-oligomer comprising Cry1Ac and Cry1F has better insertional ability than the Cry1Ac homo-oligomer complex, either during or subsequent to receptor binding. Another possibility is that the toxins Cry1Ac and Cry1F bind to different receptors in the midgut epithelium of the larvae and each individual pore made by different toxins act together and show higher toxicity than the individual pores. Receptor binding analysis and voltage clamp experiments can resolve the mode of synergism.

In conclusion, we suggest that the toxins Cry1Ac and Cry1F can be expressed together in transgenic crop plants for effective control of *H. armigera* and also as a durable resistance management strategy.

1. Kumar, P. A., Sharma, R. P. and Malik, V. S., *Adv. Appl. Microbiol.*, 1996, **42**, 1.
2. Sharma, R. P., Kumar, P. A. and Kaur, S., in *Plant Molecular Biology and Biotechnology* (eds Tewari, K. K. and Singhal, G. S.), 1997, pp. 43–52.
3. Ferre, J., Eseriche, B., Bel, Y. and Van Rie, J., *FEMS Microbiol. Lett.*, 1995, **132**, 1.
4. Tabashnik, B. E., *Annu. Rev. Entomol.*, 1994, **39**, 47.
5. Chakrabarti, S. K., Mandaokar, A. D., Kumar, P. A. and Sharma, R. P., *J. Invertebrate Pathol.*, 1998 (in press).
6. Lee, M. K., Milne, R. E., Ge, A. Z. and Dean, D. H., *J. Biol. Chem.*, 1992, **267**, 3115.
7. Laemli, V. K., *Nature*, 1970, **227**, 680.
8. Finney, D. J., *Probit Analysis*, Cambridge Univ. Press, London, 1971.
9. Tabashnik, B. E., *Appl. Environ. Microbiol.*, 1992, **58**, 3343.
10. Gill, S. S., Cowles, E. A. and Pietrantonio, P. V., *Annu. Rev. Entomol.*, 1992, **37**, 615.

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