Identification of midgut membrane proteins from different instars of *Helicoverpa armigera* that bind to Cry1Ac toxin

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Helicoverpa armigera (Lepidoptera: Noctuidae) is a polyphagous pest that feeds on important crops worldwide. This pest is controlled mainly by chemical insecticides, but also by Cry1Ac toxin produced by *Bacillus thuringiensis*(Bt). However, it was shown that larvae from late instars are less sensitive to Cry toxin action compared with early instars, reducing the efficacy of Cry toxins in the field. The most common mechanism of insect resistance to Cry toxins is linked to a reduced toxin binding to midgut brush border membranes (BBM) proteins. In this study, we evaluated the susceptibility of different larval instars of *H. armigera* to Cry1Ac toxin and correlated these data with the identification of Cry1Ac-binding proteins in to BBM vesicles (BBMV) isolated from early and late larval instars. A significant 80-fold reduction in the susceptibility of *H. armigera* to Cry1Ac toxin was observed in late instars compared to early instars. To identify the Cry1Ac binding proteins from early instars that could be responsible for higher toxicity, we compared the Cry1Ac binding proteins from second instar larvae with those of the fifth instar by pull-down assays and liquid chromatography coupled to mass spectrometry analysis (LC-MS/MS). A different protein interaction pattern of Cry1Ac toxin with BBMV proteins from the second than fifth instar was observed. Alkaline phosphatase, and other membrane proteins, such as prohibitin and an anion selective channel protein were identified only in the second instar, suggesting that these proteins may be involved in the higher toxicity of Cry1Ac in early instars of *H. armigera*. The identification and analysis of the functional role of the Cry1Ac-binding proteins in the different developmental stages of *H. armigera* will facilitate the elucidation of the mechanism of action of this toxin and may help in the control of Cry1Ac resistance evolution in this important crop pest.

Palavras-chave: Bacillus thuringiensis; Pull-down; Alkaline phosphatase

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