The vicilin variant from a resistant *Vigna unguiculata* variety (IT81D-1053) affects endocytosis in larval *Callosobruchus maculatus* midgut epithelium


It has been demonstrated that vicilin variants of cowpea seeds (*Vigna unguiculata*) are the main resistance factor present in some African genotypes against the cowpea beetle *Callosobruchus maculatus*. There is evidence that the toxic properties of these storage proteins may be related to differences between them and vicilins from susceptible in their interaction with glycoproteins and other microvillar membrane constituents along the digestive tract of the larvae. New findings, however, showed that in fact after interaction with the microvilli, the vicilins are absorbed across the intestinal epithelium and reach the internal environment of larvae and adults. Under this new perspective, the question of the mechanism of toxicity of vicilin variants was revisited. In the present paper we have studied the insecticidal activity of the vicilin variant purified from a very resistant variety, IT81D-1053. Bioassays showed that this vicilin variant affected larval growth, causing development retardation and 100% mortality. By feeding *C. maculatus* larvae on susceptible and IT81D-1053 vicilins (both of them labeled or not with FITC), followed by fluorescence and immunogold cytolocalization, we were able to demonstrate that both susceptible and variant forms are internalized in the midgut cells, migrate inside vesicular structures from the apice to the basal portion of the cells. However, when larvae were fed with the vicilin variant during 24h and returned to a control diet, the concentration of the labeled variant form remained relatively high, suggesting that the vicilin variants are absorbed, but they failed to get out of the midgut cells. We suggest that the toxic effects of vicilin variants on midgut cells of larval *C. maculatus* are not limited to the binding of these proteins to the cell surface, but the fact of being internalized and somehow interfering with their absorption process, causing a blockage of the enterocytes. The saturation of midgut cells with vicilin variant may interfere with normal nutrient absorption, thereby affecting normal larval growth *in vivo*.

**Keywords:** protein absorption; endocytosis; antibiosis; insect midgut; vicilin.

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