Abstract

Expression of *Colocasia esculenta* tuber agglutinin (CEA) in Indian mustard (*Brassica juncea* L.) with an aim to develop resistance against sap sucking insect pest

Hemipteran pest- mustard aphid (*Lipaphis erysimi*) cause huge yield loss of Indian mustard (*Brassica juncea* L.) every year. Apart from causing damage to the crop by sucking the phloem sap, this aphid also acts as vector for different plant viruses. *Colocasia esculenta* tuber agglutinin (CEA), a mannose binding lectin from the tuber of *Colocasia esculenta* has been found to be effective in controlling several hemipteran insects including *L. erysimi* in artificial diet based bioassays.

In the present study, the CEA protein was studied in detail by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The peptide homology information obtained from the LC-MS/MS analysis was used to clone the complete coding sequence of CEA and subsequent transformation into *B. juncea* cultivar cv. B-85. Southern blot analyses confirmed stable, single copy integration events of *cea* in the transgenic *B. juncea* lines. Western blot and ELISA analyses confirmed the expression of CEA. The CEA expressing *B. juncea* lines exhibited enhanced mortality (70- 81.67%) and reduced fecundity (49– 62%) of *L. erysimi* as compared to the control plants in *in planta* insect bioassays.

Biosafety assessment of transgenically expressed CEA was carried out according to the FAO/WHO guidelines (2001). Bioinformatics analysis, pepsin digestibility, thermal stability assay, targeted sera screening and allergenicity assessment in BALB/c mice model demonstrated that the expressed CEA protein from transgenic *B. juncea* plants is non- allergenic and biologically safe.

The last chapter describes the interaction between previously established mannose binding garlic leaf lectin (ASAL) and GroEL/ symbionin, a chaperonin protein synthesized by endosymbionts of aphids/ whiteflies which plays key role in virus transmission. In tomato and tomato leaf curl New Delhi virus (ToLCNDV) system the tripartite interaction between ASAL-GroEL- viral coat protein was investigated. Immunotechnique based experiments such as ligand blot, co-localization and co-immunoprecipitation demonstrated reduced rate of viral transmission by whiteflies when they are previously fed with ASAL.

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