

TRANSFER OF BT CRY GENE TO METARHIZIUM ANISOPLIAE,

A POTENTIAL EUKARYOTIC MODEL

By

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Abstract

Metarhizium anisopliae is an entomopathogenic fungus, used in biological control of insect pests that belong to the orders Lepidoptera, Coleoptera, Diptera and Homoptera. The fungus infects the host by penetrating the cuticle and spreading through the host tissues, which result in the loss of structural integrity and dehydration. However, the use of *M. anisopliae* in agriculture is limited due to its narrow host range and susceptibility to unfavorable environmental conditions. One approach to overcome these obstacles would be to use genetic engineering to incorporate favorable characters into *M. anisopliae*. But prior to any such attempt a model transformation is a must to perfect the transformation machinery. Therefore, the research was carried out to assess the feasibility of transforming *M. anisopliae* with *Agrobacterium* binary vector system. The *cry* gene of local *Bacillus thuringiensis* (Bt) strain 6e was used as the model transgene. The *cry* gene was isolated from local Bt strain 6e and cloned into *Agrobacterium* binary vector p^{ABKO1} via *Xba*I adaptor. The recombinant vector was confirmed by dot blot analysis using Dig labeled *cry* probe. Transformation of *M. anisopliae* was done by co-cultivation of the fungal spores with the recombinant *Agrobacterium tumefaciens* strain harboring Bt 6e *cry* gene cloned p^{ABKO1}. Fungal transformation was confirmed by selection on hygromycin, β -glucuronidase (GUS) assay and dot blot analysis. These results prove that the *cry* gene integrated with the *M. anisopliae* genome and the transformation machinery used is suitable for *Metarhizium*.