

Research Paper

TARGETING IMMEDIATE EARLY GENE (IE-1) FOR INDUCING VIRUS RESISTANCE AGAINST GRASSERIE DISEASE CAUSED BY BMNPV BY RNA INTERFERENCE TECHNOLOGY

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ABSTRACT

Bombyx mori L. (Lepidoptera: Bombycidae) nucleo polyhedrosis virus (BmNPV) is a highly pathogenic virus that causes grasserie infection in silkworms and effective management of the virus has been a challenge because of its sturdy nature and the lack of control strategies. RNA mediated silencing technology (RNAi) has become the tool of choice for induction of virus resistance in many organisms. A significant feature of this technology is the presence of double stranded RNA (dsRNA). In this study, Escherichia coli were engineered to produce dsRNA against ie-1(immediate early gene of BmNPV) from a plasmid (L4440) containing gene of interest under the control of double T7 promoter which efficiently produces dsRNA when it is transformed into E.coli HT115 host strain and upon induction with IPTG. Our study targeted the BmNPV ie-1 gene involved in viral multiplication. The viral copy number analysis revealed that larva fed with 50 μg of E.coli expressing ie-1 dsRNA showed lower copy number (100 copies) of the Gp41 gene against 1x10⁵ copies of Gp41 in the NPV infected silkworms. The qPCR analysis of viral genes showed 5-6-fold decrease in the viral gene expression in midguts of dsRNA fed silkworms compared to that of NPV infected larvae and showed increased survivability of > 50 % compared to infected silkworms. These results demonstrate the successful use of E.coli expressing dsRNA as an efficient and alternative tool for insect pest management.

Key words: Bioassay, BmNPV, double stranded RNA, oral RNAi, RNA interference, viral copy number.