ABSTRACT

Cry toxins are the most studied toxin than others insecticidal proteins. Once it was proved that these proteins have toxic effect and impart resistance against chewing insects of crops GM plants, a lot of experiment have conducted to evaluate the role of Cry proteins in response to insect pressure. Major class of genes encodes for these toxin proteins that are used to develop resistant GM crops against insects, to overcome the process of resistance development and to retard the mechanisms of resistance in insects, researchers suggest that overexpression of toxins in transgenic plants or transformation of more than one gene encoding insecticidal protein or should genetic transformation of combination of toxin encoding genes. The active toxins generate pores by partially inserted into the membrane; protein binds on epithelial cell of gut with specific receptors. Due to pore formation in the membrane, results in gut epithelial cell lysis that leads to death of the insects. The Cry1I proteins (Cry1Ia12) characterized and showed broad host range, these toxins are actively effect against Lepidoptera and Coleopteran. Researchers have performed bioassay and reported that heterologous Cry1Ia recombinant protein has (expressed in baculovirus) toxic effect on to S. frugiperda and A. grandis larvae. Cry1la12 gene cloned in protein expression vector pET-28 and checked the expression of CryLa12 protein in E. coli. The Cry1La12 obtained from *Bacillus thuringiensis* bacterium, this protein not shares sequence similarity with other crystal δ-endotoxin and supposed to be a possible alternate to overcome the limitations of first-generation BT protein and have broad spectrum insecticidal effect on wide range of chewing insects. The recombinant plasmid (cry1la12/pET-28) isolated from positive clones and transformed in Rosetta-gami 2 (DE3). Expression studies of eight transformants from the strain were carried out to determine the level or quantity of cry1la12 protein expression. Among the screened transformants of Rosetta-gami 2 (DE3), two clones were showing highest level of expression, in the total cell protein analysis, were checked for periplasmic expression of cry1la12. SDS-PAGE, Western blot analysis and protein quantification at OD_{280} revealed that Rosetta-gami 2 positive clone 4 and 5 showed 20% more expression of the target protein than rest of the clones. This increased expression level in Rosetta-gami 2 strain may be attributed to the presence of trxB/gor mutations which enhance disulfide bond formation and folding of the target protein, thereby improving the solubility and release of the cry1la12 in the periplasm.