ASSEMBLY OF SIX SYNTHETIC FRAGMENTS OF CRY 1 F GENE FOR Bt CROPS TO GENERATE PEST RESISTANCE IN PLANTS
Sharda Choudhary1 and P, Anand Kumar2
Department of Biotechnology, CCS University Campus, Meerut- 250 110, India.

ABSTRACT

The insecticidal protein Cry I F of Bacillus thuringiensis is highly toxic to H. armigera in a synergistic manner with Cry 1 Ac. These crystalline proteins were found to be highly toxic to agriculturally important pests at very low concentrations. However, native Bt gene expression in plant cells is sub-optimal to achieve insect protection in transgenic plants because of the presence of many mRNA destabilizing features. Cloning of fragment 6th of synthetic cry 1 F was done with Vector (pGEM7Zf) while 4th and 5th fragments were cloned with pET vector after ligation (T4 DNA Ligase enzyme). These fragments were cloned with vector pGEM carrying 6th fragment. Now fragment 1+2+3 were assembled with pGEM7Zf carrying 4th, 5th and 6th fragments. A very good band of around 1900 bp was observed which is an indication of a positive clone. Now this pGEM 7Zf vector has all the six Cry 1 F synthetic fragments in between Xba 1 and Bam H1 sites. A modified Cry I F gene was designed according to higher plant codon usage and avoiding the mRNA destabilizing features. The modified gene has 45.5% of GC content.

INTRODUCTION

Bacillus thuringiensis (Bt) is a gram-positive, aerobic, sporulating bacterium which synthesizes crystalline proteins during sporulation (Parry et al., 1983). These crystalline proteins were found to be highly toxic to agriculturally important pests at very low concentrations (Schnepf et al., 1998). Because of their lack of specificity towards mammals and other organisms, Bt strains and toxin proteins have acquired acceptability as eco-friendly biopesticides all over the world. Bt has been under extensive use in agriculture, horticulture, forestry, animal health and mosquito control for the past three decades. With the advent of molecular biology and genetic engineering, it has become possible to use Bt more effectively and rationally in transgenic plants and microorganisms (Kumar et al., 1996).

The insecticidal protein Cry I F of Bacillus thuringiensis is highly toxic to H. armigera in a synergistic manner with Cry 1 Ac. In addition, it is toxic to an important cotton pest Spodoptera litura. The gene was from the Bt subspecies aizawai. Our studies indicated that Cry I F acts synergistically against H. armigera. However, native Bt gene expression in plant cells is sub-optimal to achieve insect protection in transgenic plants because of the presence of many mRNA destabilizing features. In addition, bacterial codon usage of Bt genes needs to be changed towards higher plant codon usage to make the gene more suitable for plant expression. Hence, the present investigation was carried out at NRC Plant Biotechnology, IARI, Pusa Campus in the year 2003 for assembly of six synthetic fragments of Cry 1 F gene to generate pest resistance in plants.

MATERIAL AND METHODS

Cloning of fragment 6th of synthetic Cry 1 F was done with vector (pGEM7Zf) while 4th and 5th fragments were cloned with pET vector after ligation (T4 DNA Ligase enzyme). These fragments were cloned with vector pGEM carrying 6th fragment. Fragments 1+2+3 were assembled with pGEM carrying 4th, 5th and 6th fragments. Vector DNA and plasmid DNA were restricted for cloning of fragment and incubated at 37°C and these incubated restricted samples were checked on agarose.

The ligation mixtures were transferred to freshly prepared competent cells (CaCl2, Glycerol) to check the contamination.
colonies were observed on controlled ligation and around 12 colonies were observed on testing. Few colonies from the test ligation plate were taken and inoculated in 5 ml LB with 100 mg/l ampicillin antibiotic and kept for incubation at 37º C overnight. Numbering was given on all the tubes to identify the colonies. The well grown cultures were taken and 100 µl of the cul 3 kb was taken in autoclaved eppendorfs with 2 kb for future use before starting plasmid isolation. Plasmid isolation was done using Tris base, EDTA with RNase followed by using detergent SDS and potassium acetate, respectively. After this one litre solution of NaOH, MOPS and isopropanet was used to isolate the plasmid. Well dissolved plasmid DNA’s were checked in agarose gel for concentration. The checked DNA’s were taken for restriction to check the positive clones. The reaction mixture was kept at 37 ºC for one hour incubation. After 1 hr the samples were loaded in agarose gel and positive clones were identified. The positive clones were stored before minipreparation were taken and streaked on LA plate (100 mg/l) for further use.

RESULTS AND DISCUSSION
For final checkup the plasmid DNA from this final clone was taken and restricted with Xba 1 to Bam H 1 to see the entire assembly of 1 to 6. A very good band of around 1900 bp was observed, which is an indication of a positive clone. This pGEM 7Z vector had all the six synthetic fragments in between 1 and 6 clones.

Fig 1 Complete pGEM vector with 1 to 6 fragments of Cry 1 F gene
Analysis of Cry 1 F gene nucleotide sequence was made using Laser gene (DNAsis) program and features, which are not suitable for expression in plant cells were identified. A modified Cry 1 F gene was designed according to higher plant codon usage and avoiding the mRNA destabilizing features. The modified gene has 45.5% of GC content (Table 1). Then the gene was synthesized in six fragments and was assembled in pGEMZ. Finally it was moved into Binar, which is a good binary Agrobacterium vector for plant transformation. This synthetic Cry 1 F will be expressed in tobacco plants and the transgenic plants will be studied for insect bioassay against H. armigera and S. litura. From the bioassay results the gene will be used for different crops, which are affected by these lepidopterans.

REFERENCES


<table>
<thead>
<tr>
<th>Feature</th>
<th>Native</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT content</td>
<td>61.10%</td>
<td>53.90%</td>
</tr>
<tr>
<td>GC content</td>
<td>38.90%</td>
<td>45.50%</td>
</tr>
<tr>
<td>Poly A signals (AATAAT)</td>
<td>6.00%</td>
<td>Nil</td>
</tr>
<tr>
<td>mRNA destabilizing sequence</td>
<td>26.00%</td>
<td>Nil</td>
</tr>
</tbody>
</table>