Chapter 15

Crop Development with *Cry* Gene Constructs

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Using modern biotechnology, Monsanto has developed insect-protected Bollgard cotton and insect-protected YieldGard corn. Both of these products resulted from the transformation of a *Bacillus thuringiensis* (*Bt*) gene into the cotton and corn nuclear genomes, respectively. We discuss the development of these products in this chapter.

Two transformation methods were used to generate the transgenic events. Bollgard used the *Agrobacterium tumefaciens* method of transformation shown on the left side of Fig. 1; YieldGard was created using the biolistic gun demonstrated on the right side of the figure.

The *Agrobacterium* transformation method is well characterized and has been used for many years in the genetic modification of plant species. The plasmid vector used in plant transformation was modified so that the transformation system cannot transmit crown gall disease. The transformation system stably inserts useful genes from the plasmid vector into the chromosome of a cell. The plant material is typically wounded to allow entry of the *Agrobacterium*.

Biolistic transformation is the process in which metallic microprojectiles are coated with DNA containing the gene of interest. These microprojectiles are bombarded into the target tissue by using gunpowder, helium, or electric acceleration methods. After bombardment, the target tissue is transferred to media containing a selective agent that allows cells with the gene of interest to grow.

Bollgard Cotton

Bollgard cotton was produced using *A. tumefaciens*–mediated transfer of a variant of *Bacillus thuringiensis* subsp. *kurstaki (Bt) cry1Ac* gene, encoding a variant Cry1Ac protein that is responsible for insecticidal activity against cotton lepidopteran insect pests including bollworm, *Helicoverpa zea* (Boddie), pink bollworm, *Pectinophora gossypiella* (Saunders), and tobacco budworm, *Heliothis virescens* (F.), into the genome of Coker 312 cotton (left side, Fig. 1).

The plasmid vector within the *A. tumefaciens* used to produce Bollgard cotton contains the fully sequenced variant *cry1Ac, nptII* and *aad* genes (Fig. 2). The variant *cry1Ac* gene was derived from the common *Bt* soil microbe and encodes a variant insecticidal protein, Cry1Ac. To achieve efficacious expression levels for the variant *cry1Ac* gene, significant engineering of the nucleic acid sequence of the coding region was required to make the amino acid codons more preferable for cotton. Additional genetic elements that were added to this modified coding region include a promoter to initiate transcription, a 3′ untranscribed region to stop transcription and initiate addition of the poly adenylated end to the mRNA. This variant *cry1Ac* gene cassette contains an e-35S promoter and a 7S 3′ transcriptional termination sequence. The *nptII* gene encodes a selectable marker enzyme, neomycin phosphotransferase II (NPTII), used to identify cotton cells containing the variant Cry1Ac protein.

A large number of events were generated through transformation, which were then evaluated for expression

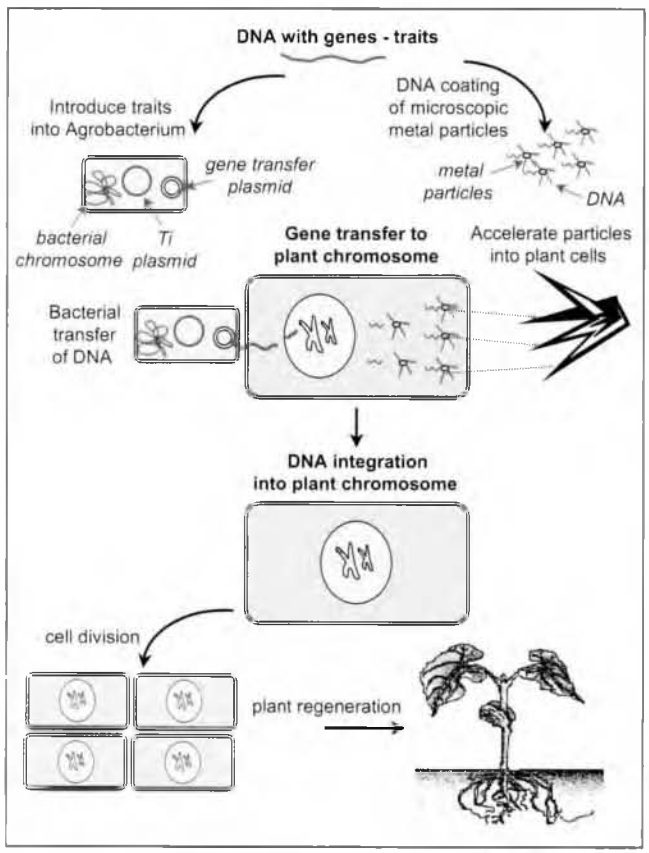

Figure 1. Transformation methods used in the generation of transgenic events in development of Bollgard cotton and YieldGard corn.