

## Transgenic Bt technology

04 February 2009 | News



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-C. Kameswara Rao Foundation for Biotechnology Awareness and Education, Bangalore, India pbtkrao@gmail.com

Transgenic technology, involving a wide range of pesticidal genes from the bacterium *Bacillus thuringiensis* (Bt), dominates the scenario of agricultural biotechnology. At the same time, Bt technology is also the most focused target of vehement anti-tech activism.

While the terms Bt cotton, Bt corn, Bt potato, etc., are familiar, the level of understanding of what the technology actually means, what it can and what it cannot do, is very poor. A variety of issues such as the biology of *Bacillus thuringiensis*, its proteins, use of Bt as a biopesticide, transgenic Bt crops, benefits and limitations of the technology and biosecurity, are important components of public awareness.

#### **Bacillus thuringiensis**

Bt is a rod shaped, gram-positive, soil bacterium, discovered in 1901. Bt is among the most thoroughly studied bacterial species of agricultural importance, its diverse aspects having been researched for over a century. The book '*Bacillus thuringiensis: Biology, Ecology and Safety*' (T.R. Glare and M. O'Callaghan, 2000, John Wiley) refers to over 8,000 research publications by over 10,000 biologists, in over 60 years, and deals with most of the issues raised against the use of Bt. Ignorance of this and other subsequent publications on Bt or a deliberate indifference to them, led to a much exploited misunderstanding of Bt technology.

#### **Concept of Bt**

The term Bt now refers to not a single simple species entity, but to a large group of subspecies and varieties, based on over 60,000 isolates, collected from all over the world. There are more than 80 serologically characterized (using specific antibodies) types of Bt.

The controversy about distinguishing *Bacillus thuringiensis* from the related pathogenic *Bacillus cereus* and *Bacillus anthracis* was adequately addressed (de Maagd, Bravo & Crickmore, July 2005). When types of Bt can be identified serologically, a microbiologist can certainly distinguish the three species.

### **Bt in nature**

Bt is a universally occurring soil bacterium, isolated from several thousand soil samples from 80 different countries. It commonly occurs also on the aerial parts of plants such as leaves and on even washed fruits and vegetables we consume. It may be present in water, possibly as a wash off from the soil and plant surfaces. Bt may be transported in the atmosphere, as inferred from its presence deep in the polar ice cap.

Bt grows and competes, but poorly in soil. Bt or its proteins may persist for about 100 days in soils, for 24 hr in running water and for 12 days in stagnant water bodies. Bt seems to require an association with plants and insects to perpetuate for longer periods in nature.

### **Bt as a biopesticide**

Bt produces a wide range of insecticidal proteins that have been in use in pest control since 1938. There are about a 100 biopesticides exclusively based on Bt and over 90 per cent of commercial biopesticides, used even in organic farming, contain Bt.

### **Bt proteins and their encoding genes**

Bt produces a large number of proteins that are toxic to specific insect groups under specific conditions. Bt also produces a) several enzymes, b) some compounds that lyse erythrocytes, and c) some that are enterotoxic to vertebrates. Bt toxins are produced either within the bacterial cell (endotoxins), or on the cell surface (exotoxins).

More than 170 toxin-encoding genes have been isolated from Bt collections. Among the endotoxins, the insecticidal crystalline proteins, called the delta-endotoxins, are significant in Bt technology. The crystalline proteins are described parasporal, as they are co-produced and co-exist along with spores (the means of bacterial propagation), in the bacterial cells. When the bacterial cell lyses to release the spores, the crystalline proteins are also routinely released into the soil.

The names of the genes that encode the crystalline proteins are prefixed with 'Cry', as for example Cry1Ab, Cry1Ac, Cry9c, etc., and the proteins that are encoded by these genes are 'Cry' proteins. The non-crystalline endotoxins are prefixed with 'Cyt'.

### **Pest specificity of Bt toxins**

Bt proteins are per se not toxic. To function as toxins Bt proteins require a specific set of biochemical and biological parameters which are available for different Bt proteins only in specific insect groups, which makes Bt toxins insect group specific. Cry1Ac and Cry2Ab control the cotton bollworm, Cry1Ab controls corn borer, Cry3Ab controls Colorado potato beetle and Cry3Bb controls corn rootworm. The Bt genes that are incorporated into different crops are specific to moths and butterflies (Lepidoptera, having wings covered by scales).

### **Pre-requisites for pesticidal activity of Bt proteins**

The following conditions are essential for an effective insecticidal activity of the Bt proteins:

- The pest must take a few bites of the plant tissue; Bt transgenics are not effective against sucking pests (Homoptera, with wings without scales), as they do not ingest plant tissue.
- An alkaline environment (pH 9.5 and above) in the gut of the insect pest is essential for the Cry proteins to dissolve in the gut fluids and to be converted into an active molecule to function as an insecticidal compound (mammalian stomachs are highly acidic).
- The pest specificity of different Bt toxins depends upon the presence of appropriate receptors, in the lining of the mid-gut (brush border) of the pest, which are absent from some pests, as evidenced by different Bt proteins being toxic to specific pest species. The toxin binds to the receptors and causes disturbance in the integrity of the gut wall, leading to leakage of the contents, followed by starvation and death of the pest.

Fundamentally, the alkaline gut environment and the presence of an appropriate toxin binding receptor are crucial for insecticidal activity of Bt proteins. Basing on such requirements, the genes that encode pest specific toxins are chosen for developing different transgenic crops.