

Role of *Bacillus thuringiensis* in development of transgenic plants

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Pest control has played a vital role in agricultural production throughout the history of agricultural societies. The introduction of various chemical pesticides has facilitated the growth of numerous crops and served as a primary pest control method. However, it has also led to challenges such as the resurgence of insect pests, the development of insect resistance, soil contamination, and environmental degradation. Concerns about food safety and pesticide-related contamination have heightened the demand for more effective and environmentally friendly pest management methods.

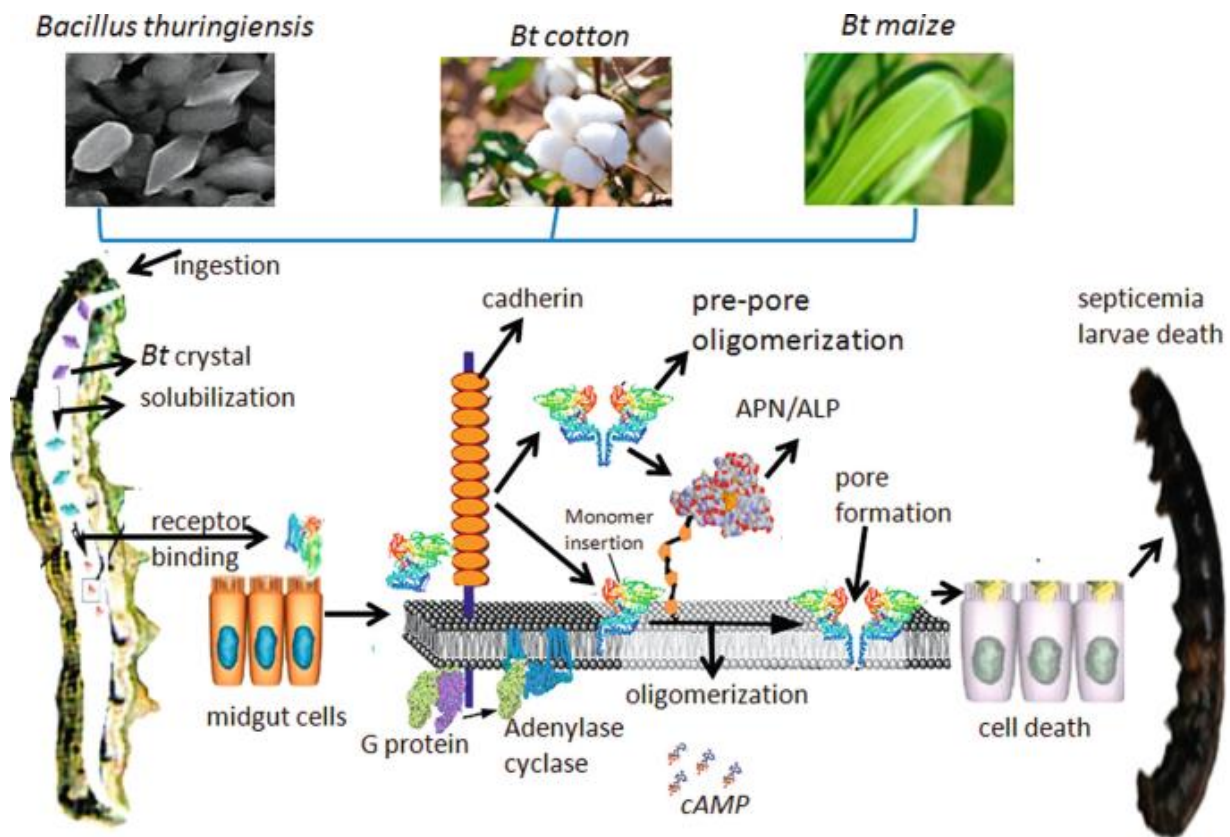
Bacillus thuringiensis (Bt) has been everlasting significance in agriculture, known for its insecticidal proteins. As a biopesticide, Bt is considered environmentally safe and versatile, extending its applications beyond insecticidal properties to act as a biofertilizer, promoting plant development. Bt, a Gram-positive, spore-producing bacterium, produces parasporal crystals in its life cycle, with these crystals containing insecticidal proteins. The bacteria's ability to generate crystal (Cry), cytolytic (Cyt), and vegetative pesticidal proteins (Vip) makes it a valuable source for developing bio-pesticides.

The insecticidal properties of Bt are attributed to the interaction of its crystalline proteins with the midgut epithelial cells of insects. Upon ingestion, the crystals dissolve in the alkaline environment of the midgut, releasing delta endotoxins, which interact with the midgut receptors and cause membrane damage, leading to the insect's death. Larval death is primarily caused by severe wounds and, occasionally, bacterial septicemia in the hemocoel. The selectivity of Cry genes depends on their ability to attach to insect midgut receptors, a crucial factor for toxicity. *Bacillus thuringiensis* stands out as an effective biological control agent commercially utilized, offering a solution to ecological and environmental concerns associated with chemical pesticides. Despite its effectiveness, challenges include the cost of using Bt as a bio insecticide and the



variability in its biological processes, depending on the strain and growing conditions. Genetically modified Bt crops, containing the crystal Bt toxin to tolerate specific insect pests, represent a significant advancement in pest control strategies. These crops, along with prepared sprays, contribute to controlling insect pests and vectors.

Bt strains like *Bacillus thuringiensis* subsp. *israelensis* (Bti) can produce both Cry toxins and cytolytic proteins. Crystalliferous mutants of Bt, such as HD73 and BMB171 strains, are employed as recipient bacteria in creating Bt engineering strains. Overall, *Bacillus thuringiensis* continues to be a valuable asset in the ongoing quest for sustainable and effective pest control measures in agriculture.



Bacillus thuringiensis in Cotton

Cotton crops face significant threats from pests, with the pink bollworm, cotton bollworm, and tobacco budworm being three prominent adversaries. Other Lepidopteran pests, such as plant bugs, aphids, and whiteflies, also contribute to hampering cotton production. To address these challenges, *Bacillus thuringiensis* (Bt) cry genes are employed. Most Cry genes, located on plasmids, control protoxin production, forming insoluble precipitates in Para crystalline bodies with a molecular weight of approximately 130 kDa during sporulation. The toxic part of Cry proteins is a 60-kDa trypsin-resistant core, connected to the N-terminal portion of the Cry protoxin. The C-terminal region of Cry proteins plays a role in the crystallization process. Upon ingestion, Bt-endotoxins disrupt the midgut epithelium of sensitive insects, leading to larval



death. The insect's alkaline midgut breaks down the crystal, releasing the Cry protoxin. Insect proteases then break down the protoxin, forming the trypsin-resistant core of the active endotoxin. The active toxin travels through the peritrophic membrane to brush border cells in the insect midgut, where it binds receptors. Integration of the toxin into the epithelial membrane creates a hole, altering membrane permeability and causing osmotic cell lysis and paralysis within minutes. Various factors, including midgut pH and protease activity, can influence the mechanism of action, thereby controlling the efficacy and specialization of a specific Cry protein. The pH-dependent solubilization of Cry proteins is impacted by the insect's midgut pH. Physiological factors and midgut pH, in turn, influence protease activity, affecting the proteolytic activation of the active endotoxin. The specificity of protease also plays a crucial role in determining insecticidal action. Receptor quantity in the membrane and the toxin's affinity for the receptor contribute to the behavior of poisonous Cry proteins. Recent studies suggest that a combination of Cry1Ac and Cry2A proves effective against lepidopterans.

***Bacillus thuringiensis* in Soya Bean**

Soybean production faces a significant challenge due to insect infestations, with *Helicoverpa armigera* larvae presenting a particularly challenging pest to control, especially as they reside in the soil. Chemical insecticides may not be effective against these soil-dwelling pests. To address this issue, cry toxins from *Bacillus thuringiensis* (Bt) have been employed in genetically engineered organisms for pest management. Several Bt genes have been inserted into transgenic soybeans to confer resistance against major insect pests. In the case of *Helicoverpa armigera*, a synthetic cry1Ac gene was inserted into the soybean variety "Jack," resulting in a transgenic lineage known as Jack-Bt. This transgenic lineage exhibited three to five times less earworm defoliation compared to untransformed soybeans. Additionally, research has shown that transgenic soybeans, including Jack-Bt, exhibit some level of resistance to another pest, *Helicoverpa parallela*.

Insecticidal activity against lepidopteran pests has been a consistent outcome in various studies on Bt transgenic soybeans. Moreover, recent research has explored the introduction of exogenous cry8-like genes into soybeans, successfully providing resistance against *Helicoverpa parallela*. This demonstrates the potential for enhancing insect resistance in soybeans through genetic modification, contributing to more sustainable and resilient soybean cultivation practices.

***Bacillus thuringiensis* in Potato**

The Colorado potato beetle poses a significant threat to potato plants globally, with both adults and larvae causing damage to the leaves. This damage is characterized by holes of various sizes, often starting at the leaf margins, and can lead to defoliation. In addressing this issue, *Bacillus thuringiensis* (Bt) strains, known for their insecticidal properties, have been employed



as foliar sprays against a variety of pests. One specific strain, *Bacillus thuringiensis* var. *tenebrionis*, produces a parasporal crystal protein called Cry3A, which exhibits insecticidal properties against the Colorado potato beetle. Cry proteins are the primary active components in Bt-based microbial insecticides. Cry3A has high unit activity and is selectively effective against various coleopteran insect pests, making it particularly useful in controlling the Colorado potato beetle.

The advantage of Bt insecticides lies in their specificity for target pests, such as the Colorado potato beetle, while causing minimal harm to humans, unintended wildlife, or beneficial arthropods. Bt insecticides serve as a significant alternative to traditional chemical pesticides, especially in integrated pest management programs. Additionally, Bt sprays have the benefit of being photosensitive and rapidly decomposing, providing plant protection with minimal environmental impact compared to many chemical insecticides.

Crop	Bt Toxin	Pest	Effect
Cotton	Cry1Ac	<i>Helicoverpa armigera</i>	Effective
Maize	Cry1Ab	Corn borer	Effective
Soya Bean	Cry1Ac	<i>Spodoptera litura</i>	Effective
Potato	Cry3A	<i>Leptinotarsa decemlineata</i>	Effective
	Cry1Ab	<i>Pthorimaea operculella</i>	Non-Effective
Tomato	Bt strain 4D1	<i>Tuta absoluta</i>	Effective
Rice	Cry1F	<i>Helicoverpa armigera</i>	Effective
Sugarcane	Cry8	<i>Holotrichia serrata</i>	Effective
Brinjal	Cry1Ac	Shoot borer	Effective
Cabbage	Cry1B	<i>Plutella xylostella</i>	Effective
Cauliflower	Mixture of Cry1Ab with Cry1 and Cry2 toxins	<i>Plutella xylostella</i>	Effective

Conclusion

The discovery of *Bacillus thuringiensis* (Bt) genes is discussed as a significant development in crop protection strategies. The introduction of Bt genes in crops, such as Bt crops, has opened up new avenues for enhancing crop resistance to pests. The text mentions a promising strategy for boosting crop resistance, involving host-mediated RNA interference (RNAi) targeting crucial pest genes. This approach alters plants to produce double-stranded RNA against specific target genes in pests, resulting in decreased pest resistance to insecticides and limitations on pest growth and reproduction. The effectiveness of this strategy is highlighted by the successful suppression of target pest viability and egg production through CRISPR/Cas9-mediated deletion of associated genes. Additionally, the passage provides information about the diversity of genes



involved in insecticidal properties. It mentions 146 VIP genes classified under four families, 40 Cyt genes classified in three families (Cyt 1, Cyt 2, and Cyt 3), and over 800 cry genes categorized into 75 families (Cry1 to Cry75), along with specific details about the targets of these genes. The toxic effects of these genes on various pests, such as butterflies, moths, flies, mosquitoes, beetles, weevils, Dipterans, and nematodes, are noted with insecticidal properties, emphasizing the ongoing exploration and discovery in the field of crop protection and genetic modification for pest resistance.

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