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Role of *Bacillus thuringiensis* in development of transgenic plants

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Abstract

One of the most significant advancements in plant biotechnology has been the production of genetically engineered plants. Due to the effects of pests damaging the majority of crops, the development of pest immunity was necessary for crop preservation. Plants that have had their gene makeup altered in-utero, such as *Bacillus thuringiensis*, which has insecticidal properties and helps protect crops from pests, are referred to as "genetically modified plants." Cry proteins, which are poisonous proteins that exist in the state of crystals, are the major genes responsible for the development of transgenic plants. Based on the effect of different pest species, cry proteins are divided into many categories. Since they are extremely specific by nature and only affect the target proteins, they are considered environmentally beneficial pesticides since they have no impact on the physiologically significant soil bacteria or other bacterial flora. These cry proteins stay as dormant crystals, but when a pest consumes plants, the inactive form of the crystals becomes active in the alkaline stomach pH of the microorganism, aiding in the rupture of the gut epithelium and ultimately causing the microorganism to die. These days, transgenic plants have been created, including BT corn, BT rice, sugarcane, brinjal, potato, tomato, and many more, it was also discovered that using these transgenic plants increased crop productivity. Transgenic plants can prevent several ecological issues associated with traditional pesticides, including the emergence of resistance, their toxicity to non-target living things, and the buildup of toxic waste in the environment.

Keywords

Transgenic plants; *Bacillus thuringiensis;* Cry proteins; Biopesticide; genetically modified plants

Introduction

Pest control has long been a crucial component of agricultural production in agricultural societies. The introduction of different chemical pesticides has encouraged the development of many crops and served as the primary pest control measure. Additionally, it brought up significant issues like the resurgence of insect pests, the emergence of insect resistance, soil contamination, and environmental degradation. Problems with food safety and pesticide-related contamination have increased demand for more efficient and secure pest management methods (1).

Bacillus thuringiensis (Bt) has also been used in agriculture for a long time since it contains insecticidal proteins. This makes it a useful biopesticide that is safe for the environment. Its applications are not however limited to those that are insecticidal. It can act as a biofertilizer to promote plant development (2). *Bacillus thuringiensis* (Bt) is a common Gram-positive, spore-producing bacteria that, after its life cycle, generates parasporal crystals (Fig.1). Bt is commonly utilized to create bio-pesticides because the parasporal crystal proteins have insecticidal properties (3). The bacteria Bacillus thuringiensis produces crystal (Cry), cytolytic (Cyt), and vegetative pesticidal proteins (Vip), each of which is harmful to different insect groups (4). The crystal, which was ingested by an insect that feeds on plants, dissolves in the midgut's alkaline environment, releasing one or more insecticidal crystalline proteins also referred to as delta endotoxins, additionally, protease specificity may be a critical factor in determining insecticidal action. They interact with the midgut epithelial cells, causing membrane integrity to be destroyed and eventually death (Refer Fig 2) (1). Serious wounds and, occasionally, bacterial septicemia in the hemocoel are the main causes of larval death. The selectivity of the Cry genes is determined by their ability to attach to the midgut receptors of insects, which is a necessary prerequisite for toxicity. Several proteins, including aminopeptidases, glycolipids, cadherins, alkaline phosphates, and ABC transporters, have been discovered to date as Bt receptors in Lepidoptera (5).

Among the most effective biological control agents utilized commercially is *Bacillus thuringiensis* (Bt). The ecological and environmental issues brought on by the usage of chemical pesticides may be lessened by its products. Two of Bt's limitations include the expense of using it as a bio insecticide and the difficulty of ensuring its biological processes, which might vary depending on the strain and growing conditions (6). Bt crops are plants that have been genetically modified to contain the crystal Bt toxin to be tolerant of specific insect pests (7). Two examples of secreted proteins that build up in parasporal crystals during the sporulation phase are the Cry and Cyt proteins. The vegetative stage of growth involves the production of more proteins (5). Currently, prepared sprays and transgenic crops that contain insecticidal proteins from various classes are used to control insect pests and vectors (8). Some Bt strains, including *Bacillus thuringiensis* subsp. *israelensis* (Bti), can manufacture both the Cry toxins and the toxic crystal known as cytolytic protein, or Cyt toxin (7). Recently, some Bt crystalliferous mutants, such as the HD73 and BMB171 strains, have been used as recipient bacteria to establish Bt engineering strains (9).

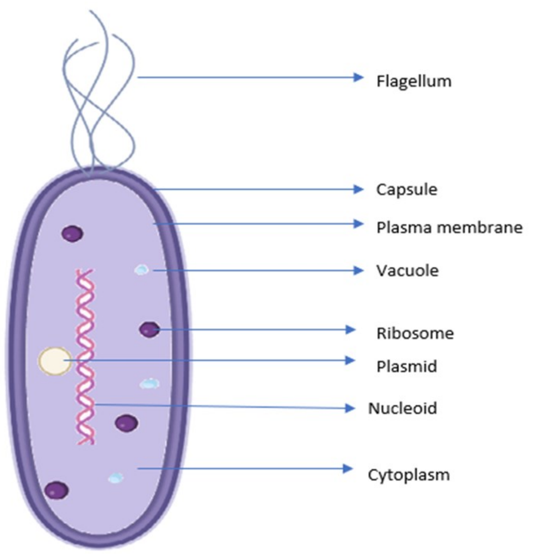

Fig 1. Structure of *Bacillus thuringiensis*

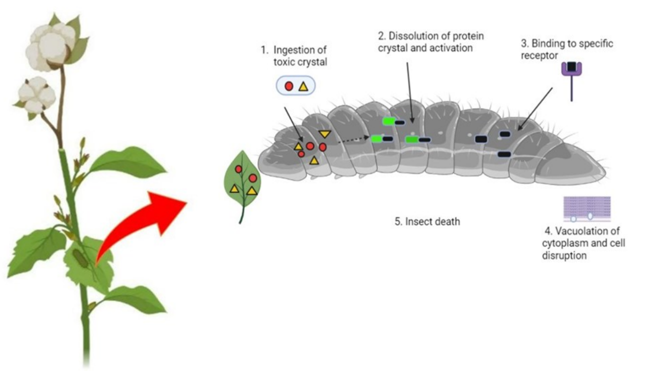

Fig 2. Action mechanism of Bt toxin

Bacillus thuringiensis In Cotton

Three significant pests that have harmed cotton crops include the pink bollworm, cotton bollworm, and tobacco budworm. The production of cotton is hampered by other Lepidopteran pests as well, including plant bugs, aphids, and white flies. *Bacillus thuringiensis* cry genes are employed to combat this issue. The majority of Cry genes found on plasmids control the production of protoxins, which are accumulated in Para crystalline bodies as insoluble precipitates upon sporulation and have a molecular weight of about 130 kDa. The poisonous moiety of Cry proteins is limited to a 60-kDa trypsin-resistant core that connects with the N-terminal portion of the Cry protoxin, whereas the C-terminal region of Cry proteins is involved in the crystallization process. After ingestion, the midgut epithelium of sensitive insects is disrupted by the Bt -endotoxins, which results in larval death. The insect's alkaline midgut breaks down the crystal, releasing the Cry protoxin in the process. Insect proteases subsequently break down the protoxin to produce 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. By further integrating the toxin into the epithelial membrane, a hole is formed that changes the permeability of the membrane and results in osmotic cell lysis and paralysis within minutes. Numerous variables can readily affect the mechanism of action, which in turn controls the action and specialization of a given Cry protein. Because Cry protein solubilization is pH dependent, the pH of the insect's midgut can have an impact. The physiological factors and the midgut pH in turn control protease activity and predictably have an impact on the active endotoxin's proteolytic activation. Additionally, the specificity of protease itself may be a key factor in determining insecticidal action. The quantity of receptors in the membrane as well as the toxin's affinity for the receptor play a role in how poisonous Cry proteins behave (10). Recent studies show that a combination of Cry1Ac and Cry2A are effective for lepidopterans (11). It was also observed that some transgenic cotton varieties had no lethal or sublethal effect on pests in laboratory conditions (12).

Bacillus thuringiensis in Soya Bean

A significant obstacle to soybean production is insect infestations. Because they live in the soil, larvae of *Helicoverpa armigera* are one pest that is challenging to eradicate using chemical insecticides. Since the poisonous effects of cry toxins are predicated on the rupture of midgut cells inside insect pests, they have been frequently exploited in genetically engineered organisms for pest management. Multiple Bt genes have been inserted into transgenic soybeans to confer resistance against significant insect pests of crops. Previous research on the insecticidal activity of soybean plants revealed that the cry gene had been inserted into the plants. A transgenic lineage of the soybean variety "Jack" that produces a synthetic cry1Ac gene (Jack-Bt) showed three to five times less earworm defoliation as compared to untransformed soybean. According to a study, transgenic soybean also has some resistance to the pest. *Helicoverpa parallela*. (Table 1). Insecticidal action against lepidopteran pests has been demonstrated in the majority of investigations on Bt transgenic soybeans. It also demonstrated the viability of producing insect resistance in soybeans by introducing an exogenous cry8-like gene. Recent research has successfully integrated cry-8-like genes into soybeans to provide them resistance to *H. parallela* (13).

Bacillus thuringiensis in Potato

Globally, the potato is a significant crop. The primary pest of potato plants is the Colorado potato beetle. Adults and larvae of the Colorado potato beetle attack potato plant leaves, causing damage in the form of holes of various sizes, which typically begin at the margins. A network of veins as well as petioles is frequently left behind after the leaf edges are eaten. Defoliation may result from this. *Bacillus thuringiensis* strains have been applied as foliar sprays against a variety of pests to address this issue. Cry proteins are the primary active components of Bt-based microbial insecticides, which have been used as foliar sprays in agriculture for many years. *Bacillus thuringiensis* var. tenebrionis produces a parasporal crystal protein known as Cry3A that has insecticidal properties against the Colorado potato beetle. This protein has high unit activity and selectivity for various coleopteran insect pests, including the Colorado potato beetle. The benefit of Bt insecticides has been that they typically do not damage people, unintended wildlife, or helpful arthropods. It is a significant substitute for traditional chemical pesticides in several integrated pest management programs because of its distinct mode of action and selectivity. In contrast to the majority of chemical insecticides, Bt sprays' poisons are photosensitive and decay fast, thus their application offers only minimal plant protection (14).

Table 1. Different crops with respective Bt toxic protein for specific pests.

Crop	Bt Toxin	Pest	Effect	Reference
Cotton	Cry1Ac	*Helicoverpa armigera*	Effective	(1)
Maize	Cry1Ab	Corn borer	Effective	(19)
Soya Bean	Cry1Ac	*Spodoptera litura*	Effective	(1)
Potato	Cry3A	*Leptinotarsa decemlineata*	Effective	(1)
	Cry1Ab	*Pthorimaea opercullela*	Non-Effective	
Tomato	Bt strain 4D1	*Tuta absoluta*	Effective	(20)
Rice	Cry1F	*Helicoverpa armigera*	Effective	(1)
Sugarcane	Cry8	*Holotrichia serrata*	Effective	(21)
Brinjal	Cry1Ac	Shoot borer	Effective	(22)
Cabbage	Cry1B	*Plutella xylostella*	Effective	(23)
Cauliflower	Mixture of Cry1Ab with Cry1 and Cry2 toxins	*Plutella xylostella*	Effective	(24)

Advantages and disadvantages of transgenic plants

By adding beneficial artificial genes or preventing the expression of native genes in crop plants, transgenic crops, and genetically modified plants have played crucial roles in crop improvement. Bt cotton is primarily grown for use in garments, the production of edible oil, and animal feed. The deployment of Bt-cotton technology over the past 24 years has produced numerous financial gains. The use of chemical sprays in the field to combat insects has dropped since its adoption; as a result, it has had a good impact on both the environment and the health of farmers and customers. To produce ripened tomatoes without them becoming soft and with a longer shelf life, genetically engineered tomatoes were created. Additionally, genetically modified plants provide crop protection, pest resistance, and environmental sustainability (15). According to past research, genetically modified organisms cut pesticide consumption and greenhouse gas emissions. By decreasing these harmful substances, which include greenhouse gases and pesticides, it opens the door for overall sustainable development (16).

The introduction of genes into genetically modified plants has the potential to spread to unrelated plants or even to other creatures in the ecosystem, which results in genetic contamination. Additionally, it was noted that there is a minimal risk to human health (17). The introduction and production of genetically modified plants without proper regulation thus pose a serious risk to the biodiversity and genetic variety of native plants owing to "biological contamination". A scientific strategy must thus be used to control the monitoring and growing of genetically modified plants in the fields because they are of unique value. This tactic would enable the preservation of the genetic diversity of wild plant communities while excluding agroecological and environmental dangers (18).

Results And Discussion

Agriculture is a significant source of revenue; thus, it is crucial to safeguard the crop against pests. With several insecticidal methods that can destroy the intended pests, the discovery of the Bt genes opens the door for significant directions in the development of Bt crops. A promising strategy for boosting crop resistance to pests is host-mediated RNAi of crucial pest genes. Plants that have been altered to produce double-stranded RNA against appropriate target genes in pests have successfully decreased pest resistance to insecticides or restricted pest growth and reproduction. It has been demonstrated that target pest viability and egg production are efficiently suppressed by CRISPR/Cas9-mediated deletion of associated genes (1). There are 146 VIP genes classified under 4 families, 40 Cyt genes classified in 3 families (Cyt 1, Cyt 2, and Cyt 3), and more than 800 cry genes found and categorized into 75 families (Cry1 to Cry 75). (Vip 1 to Vip4). The CryI toxins are poisonous to butterflies and moths, the CryII genes are poisonous to flies and mosquitoes, the CryIII genes are poisonous to beetles and weevils, the CryIV genes are poisonous to Dipterans, and the CryV is nematode-active toxins. Further research may uncover many other genes.

Conclusions

Plants that have undergone genetic modification are essential to the advancement of agriculture. Pests pose the biggest danger to agriculture, but they can be managed with the help of transgenic plants. Many ecological problems linked to conventional pesticides can be avoided by using transgenic plants, such as the development of resistance, toxicity to organisms other than intended targets, and accumulation of hazardous waste in the environment. They are regarded as ecologically helpful pesticides as they exclusively disrupt the target proteins and are incredibly selective by nature, leaving unaffected by the physiologically important soil bacteria and other bacterial flora.

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Authors' contributions

RCS devised the article, searched the literature, formatted the manuscript, and plotted figures. MP, AVA, BB, and AM revised the work. CCM, KP, and AC searched and supplemented the literature. MP participated in the design and coordination of the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors attest that they have no financial or personal ties that might be interpreted as endangering the findings of the study that was submitted.

Ethical issues: None.

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