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Mini Review

The use of *Bacillus thuringiensis* to control plant-parasitic nematodes

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Abstract

Plant-parasitic nematodes are ubiquitous in nature and cause large losses in agriculture. The current concerns regarding the use of chemical pesticides have increased the interest in new control alternatives. One of these is the one based on Bacillus thuringiensis (Bt). These Gram-positive bacteria have the ability to synthesize pesticide proteins during sporulation. Some of these proteins have nematicidal properties. Studies have shown that preparations of certain strains of Bt can prevent or slow down the infestation of phytonematodes. The expression of some Bt nematicidal genes in transgenic plants has also demonstrated their effectiveness. Bt is nowadays an effective ecological alternative for controlling plant-parasitic nematodes.

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Nematodes are the dominant fauna in most soil communities [1]. There are more than 4100 species of plant-parasitic nematodes, which cause severe damage to crops all around the world. The magnitude of the losses depends fundamentally on population densities in soil and roots, susceptibility of the crop, and environmental conditions such as the temperature of the soil, which largely affect the development of nematodes. Some plant-parasitic nematodes cause severe crop losses to food and fiber crops [2], annually estimated to be US $78 billion worldwide [3]. In the Mediterranean area, production losses by the root-knot nematodes on the horticultural crops have been estimated to be between 15 and 60% [4].

Plant-parasitic nematodes are hidden and unseen enemies of crop plants as they are microscopic and are present in the soil. They can be grouped as (A) Sedentary endoparasites, the cyst and root-knot nematodes, the most damaging obligate plant-endoparasitic nematodes affecting a wide range of plant species worldwide (Figure 1) [5]; (B) Semi sedentary endoparasites, (C) Migratory endoparasites

Figure 1: Bt and root-knot nematode. Bacillus thuringiensis spores and crystals (A), J2 stage of Meloidogyne javanica (B), and tomato roots affecte by root-knot nematodes (C).

(D) Virus transmitter nematodes, and (E) Stem and leaf damaging nematodes. The plant-parasitic nematodes that are considered to cause the greatest social and economic impacts are: root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* and *Globodera* spp.), root-lesion nematodes (*Pratylenchus* spp.), the burrowing nematode *Radopholus similis*, the migratory endoparasite *Ditylenchus dipsaci*, the pine wilt nematode *Bursaphelenchus xylophilus*, the reniform nematode *Rotylenchulus reniformis*, the virus vector nematode *Xiphinema index,* the false root-knot *Nacobbus aberrans,* and *Aphelenchoides besseyi,* an important pathogen of rice [5]. In general, nematode infection results in above and ground symptoms in plants, like general plant wilting, leaf necrosis, chlorosis, leaf dropping, stunted growth, and in the case of penetration to the root systems, root gall and knot formation. Furthermore, nematicidal infection results in enhanced susceptibility to other pathogens [6].

The necessity of increasing the food (crop) production by at least 2% every year to assure an appropriate world food supply [7] drives the search for agricultural protection systems against pests while preserving the environment and avoiding the accumulation of chemical residues in nature. *Bacillus thuringiensis* (Bt), is a well-known entomopathogen. It is a Gram-positive ubiquitous bacterium distributed worldwide. One of its most known characteristics is that during sporulation it produces parasporal crystals composed of pesticide proteins (Figure 1) [8,9]. This bacterium is safe for other organisms, including humans [10]. Therefore, Bt-based compounds have been used as the most successful microbial insecticides for decades [11,12] and it is one of the most promising biological control agents.

The pesticide proteins produced by Bt are toxic to larvae of many insect species and also to nematodes [9,13,14]. To exert its toxic action, it is widely accepted that the Bt pesticidal proteins included in the crystalline inclusion bodies have to be solubilized in the midgut of the susceptible organism. After activation and binding to specific midgut membrane receptors, several processes can happen, such as the activation of intracellular death pathways [15]. Additionally, or alternatively, the sequential biding model processes can take place: oligomer promotion, insertion in the membrane, and pore formation that break the epithelial cells and also allow the bacteria to infect hemocoel causing septicemia [16,17].

The toxicity of Bt to nematodes is well established since 1972 when the first study showing the toxicity of Bt against *Meloidogyne* spp. was published [18]. Seven classes of Bt Cry toxins have been reported to have activity against nematodes: Cry5, Cry6, Cry12, Cry13, Cry14, Cry21, and Cry55 [14,19,20]. In addition, other Bt proteins apart from Cry (such as thuringiensin, chitinase, and metalloproteinases) are toxic to nematodes (as a review, see [19,21]) which can increase its effectivity. As an additional beneficial effect, Bt can also promote plant growth [22-24]. The mode of action of Bt Cry toxins in nematodes is not well established, but it is known that carbohydrates are essential for Cry5B toxicity to *Caenorhabditis elegans,* to allow binding and therefore toxicity [25], that cadherin acts as a receptor [26] and that Bt also targets the intestinal epithelial junctions in this organism [27].

The effectivity of Bt in controlling plant-parasitic nematodes such as *Meloidogyne hapla* has been reported, after soil drenching with spore-crystal mixtures of Cry6 in tomato plants, decreasing galling index and egg masses on host root, and reducing the final number of nematodes in soil [28]. Similar results have been obtained against *Meloidogyne incognita* after treating tomato plants with Bt strains, which were also able to translocate into the plant tissues [29]. The susceptibility of *M. incognita* to Cry5, Cry6, and Cry55 proteins is well established [30,31]. Additionally, it has been published that *Meloidogyne javanica* infestation was reduced after Bt treatment [32].

Apart from the conventional use of Bt for crop treatments, transgenic Bt crops expressing Lepidopteran and Coleopteran active proteins have been developed and commercialized since 1996. The Bt crops can control successfully the target pests and are planted in several countries in the world since the last years of the past century ([33], https://www.isaaa.org/gmapprovaldatabase/default.asp, last accessed 2nd June 2022). Similarly, the gene coding for Cry5B protein has been transformed in tomato plants and in the fungus *Botrytis cinerea* to control *M. incognita* and the pine wood nematode *Bursaphelenchus xylophilus* respectively [34,35] with successful results in the control of the two phytonematodes. Also, it has been reported that tomato roots expressing Cry6A decreased *M. incognita* population [36] and soybean transformed with the Cry14 gene showed a reduction of soybean cyst nematode *Heterodera glycines* adults and eggs [37]. Indeed, the US Environmental Protection Agency recently approved the registration of transgenic soybean GMB151 targeting the soybean cyst nematode (https://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=562, last accessed 2nd June 2022).

Summarizing, Bt strains or Bt Cry proteins can be excellent nematicidal agents that can be part of new generation strategies for the control of plant-parasitic nematodes.

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