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Review

Belowground environmental effects of transgenic crops: a soil microbial perspective

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Abstract

Experimental studies investigated the effects of transgenic crops on the structure, function and diversity of soil and rhizosphere microbial communities playing key roles in belowground environments. Here we review available data on direct, indirect and pleiotropic effects of engineered plants on soil microbiota, considering both the technology and the genetic construct utilized. Plants modified to express phytopathogen/phytoparasite resistance, or traits beneficial to food industries and consumers, differentially affected soil microorganisms depending on transformation events, experimental conditions and taxa analyzed. Future studies should address the development of harmonized methodologies by taking into account the complex interactions governing soil life.

Keywords: Microbial communities; Transgenic plants; Pleiotropy; Horizontal gene transfer; *Bt* plants

1. Introduction

The cultivation of transgenic plants (or genetically modified plants, GMPs) has prompted scientists to seek greater understanding of their direct and indirect impact on natural and agricultural ecosystems. While GMPs have been assumed to be safe in terms of human health, unforeseen environmental effects have been observed in the field, varying according to the genetic traits of the modified plants, and in space and time, as a result of the complex network of interactions ruling aboveground and belowground ecosystem functioning [1]. Some of the effects reported in the available scientific literature may be directly ascribed to the technology utilized, while others are linked to the nature of the genes introduced in the transgenic plants.

Most transgenic events have been obtained using the cauliflower mosaic virus (CaMV) 35S RNA promoter, which induces constitutive expression of transgenic proteins: some of them act as toxins towards particular groups of organisms and are exuded by the roots [2–4]. This stresses the need to assess the effects of such genetic modification on microbes living in the rhizosphere and in the soil. In such environments plants release up to 25% of the carbon allocated to the roots as root exudates [5], and crop residues are incorporated at the end of production cycles. Other outcomes of the technology used for production of transgenic plants may derive from pleiotropy, a phenomenon leading to development of unexpected phenotypes as a result of insertions of foreign genes in a new genomic context. For example, some GMPs showed increases or decreases in the content of plant secondary metabolism compounds or alterations in crop chemistry not directly linked to the particular genes introduced [6–8], which might affect, directly or indirectly, the soil microbiota.

With regard to the nature of the genes introduced in transgenic plants, the use of marker genes for antibiotic

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resistance and their fate during and after cultivation in the field have been considered critical issues by the World Health Organization [9], as antibiotic resistance genes may be transferred to rhizosphere and soil microbes, and from them to pathogenic bacteria, through horizontal gene transfer (HGT) [10]. In addition, crops modified to tolerate broad-spectrum herbicides like glyphosate have also raised concerns, as glyphosate inhibits Class I EPSPS, a key enzyme in the synthesis of aromatic amino acids occurring in plants, fungi and bacteria [11].

GMPs may directly or indirectly impact the structure, function and diversity of soil and rhizosphere microbial communities, which play key roles in the belowground environment, providing essential ecosystem services, e.g. decomposition of crop residues, completion of biogeochemical cycles within the soil food web, and maintenance of environmental quality and productivity [5]. Rhizosphere microorganisms may be affected by plant genotype [12] and by changes in agricultural management inherent to cultivation of transgenic plants, such as herbicide application. Thus, they represent potential key non-target organisms to be monitored in studies on the environmental impact of transgenic crops (Fig. 1).

In this work, we review available data on direct, indirect and pleiotropic effects of GMPs on the structure and function of soil microbial communities, considering both the technology utilized for production of engineered plants and the nature of the transgenes.

2. Direct, indirect and pleiotropic effects of transgenic plants on soil microbes

2.1. Transgenic plants constitutively producing Bt toxins

Bt plants are engineered with *cry* genes derived from the soil bacterium *Bacillus thuringensis* Berliner to express insecticidal δ-endotoxins (called crystal proteins or Cry proteins), conferring resistance to some insect pests from the orders Lepidoptera, Coleoptera or Diptera [13]. The amounts of *Bt* toxins expressed in plant tissues and released into the environment, ranging from 152 to 183 ng per gram in decomposing root residues, directly derive from the technology utilized to produce transgenic plants constitutively expressing Cry proteins. Such data will deserve attention in the years to come, in particular in multiple *Bt* toxin stacked-trait lines [14]. Indeed, it has long been known that insecticidal *Bt* toxins are exuded by *Bt* maize roots into the soil [2] where, together with those derived from plant residues, they are bound to humic acids and clay soil particles and, protected from microbial degradation, often maintain their activity [13]. Some authors reported that the Cry3Bb and the Cry1Ac toxins may persist for 21 and 56 days in soil microcosm and laboratory experiments, respectively [15,16], and that no *Bt* toxin is retrieved from field soils for 3–6 consecutive years of *Bt* cotton cultivation [17]. Variable persistence has been observed for the Cry1Ab toxin, which was not detected in a nine-year field trial of *Bt*-maize MON810 [18], or else was shown to be still detectable after 4 years in the field [13], possibly depending on soil chemical and physical characteristics.

In an experiment carried out on *Bt* maize plants in relation to soil biota, Saxena and Stotzky found that the CryIAb toxin released into root exudates or directly incorporated into soil exerted no adverse effects on culturable bacteria or saprophytic fungi (nor on earthworms, nematodes or protozoa) [19]. Small or no changes in culturable microflora were detected in the rhizosphere of Cry-expressing cotton and rice and in the composition of microbial communities in the presence of Cry1Ab maize residues compared with control plants (Table 1). Accordingly, two long-term field studies found no consistent differences in soil microbial communities between GMPs and controls or during successive years [15,20]. A significant temporary decrease in saprophytic fungal populations was observed 30 days after sowing *Bt* maize in comparison with the isogenic line [21], and variation in fungal decomposer communities was detected in one out of 16 trials by Xue et al. [22] (Table 1).

Other works, using culture-independent methods, reported no significant or only slight effects of *Bt* maize plants on soil microbial communities, suggesting that plant age, soil type and texture may represent the overriding factors affecting bacterial diversity (Table 1). In contrast, differing fingerprints of soil bacterial communities exposed to *Bt* maize were reported by other authors [23–26]. Castaldini et al. [25] also observed that microbial activity, assessed by measuring soil respiration, changed in soils amended with *Bt* plant residues, in agreement with other reports [27,28] (Table 1).

In the majority of the cited studies, it is impossible to distinguish between effects that can be directly ascribed to the toxins and indirect and non-specific outcomes of transgenic events (pleiotropy). However, an interesting work highlighted the occurrence of pleiotropic effects which were not linked to the products of the inserted genes, but resulted from transformation technology [16]: the cultivation of *Bt* cotton affected soil microbial populations, while the purified *Bt* toxin showed no effect. These data were corroborated by results detailed in Naef et al. [29], who found that purified Cry1Ab toxin did not inhibit growth of *Fusarium graminearum* or *Trichoderma atroviride*, while *Bt* and non-*Bt* maize residues affected fungal growth in vitro (Table 1).

A pleiotropic effect of *cry1Ab* transgenic plants - alteration in the shikimic acid pathway leading to a higher lignin content in the stem - was detected in several transformation events of *Bt* maize lines [6,8] and also in *Bt* canola, cotton, potato, rice and tobacco [27]. However, the harm or benefits of the slower degradation rate of *Bt* plant residues and putative resulting shifts in microbial community composition remain to be verified. A field study [30] found that *Bt* maize decomposed significantly faster than non-*Bt* maize in winter in bags with 20 and 125 μm mesh sizes, which excluded macrofauna but allowed microflora (bacteria, fungi) and mesofauna activity. Such results were explained by the higher amount of proteins in the plant matrix (20% of *Bt* toxin still present), which stimulated growth of soil microbial populations. Conversely,