

Impact of transgenic *Bt* cotton on soil health

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Abstract

Cotton is an important fibre crop of global and economic significance. For effective control of bollworms, transgenic *Bacillus thuringiensis* (*Bt*) cotton was introduced on a commercial scale in 1996, with an aim to reduce pesticide consumption. Presently, transgenic cotton is grown on 24 million hectares, which occupies 14% of global acreage planted to transgenic crops (185.1 million hectares). In recent years, considerable research efforts have been directed towards the study and assessment of the use of transgenic crops. Although transgenic *Bt* cotton is proven for its benefits in controlling insects, concerns still remain about its ecological impact on soil ecosystem function and biodiversity. This review focuses primarily on the persistence of *cry* toxin, if any, and impact on soil ecosystem functioning; because sustainable agriculture depends on soil – the basic natural resource. Based on the research from cotton-growing nations, there was no solid substantiation that points out adverse effects on soil health or fertility in terms of soil biology and ecology following cultivation of transgenic cotton. The *Cry* proteins released through root exudates and plant residues of *Bt* cotton appears to have no consistent and long-term effects on the soil biology. Some differences between *Bt* and non-*Bt* cotton were indicated for soil microbial community structure and their population. However, majority of the studies indicated that these differences were transient in nature and not statistically significant. Therefore, differences observed may not be related to the inserted *Bt* transgenes.

Keywords: *Cry* toxin, *Gossypium* sp., Soil fertility, Soil enzymes, Microbial diversity

Review Methodology: For this review, we gathered information on transgenic *Bt* cotton and its effect on soil properties from published research, reviews and reports from various sources including CAB Abstracts.

Introduction

Soil is the basic natural resource, which serves as a medium for all living things including plants. Globally, therefore, studies are directed to preserve the soil in its natural form with eco-friendly technologies in order to sustain agricultural productivity and environmental quality [1–4]. Soil composition reportedly affects crops, their quality, nutritional value and human health [5]. Recently, soils under intensive cultivation started showing a decline in production, either due to non-replenishment of plant nutrients [6] or adverse climate [7]. Although soil quality and soil health are used interchangeably [8], they are not the same. Soil quality is associated with soil function and mainly concerned with the physical and chemical properties [9, 10], whereas soil health presents soil as non-renewable

and active living resource [11]. A number of indices were proposed [12–15], such as physical, chemical and biological parameters that influence crop productivity and soil fertility in one way or the other (Figure 1).

Transgenic crops were first introduced for widespread cultivation in 1995. Within a span of two decades, acreage planted to transgenic crops increased 110-fold from 1.7 million hectares in 1996 to 185.1 million hectares in 2016 [16]. This rapid increase in growth was due to the proven benefits of transgenic crops with regard to control of pests and diseases, crop yield increase, enhanced vitamin expression and reduced usage of plant protection chemicals. However, there are concerns about the possible negative impact of transgenics on soil biodiversity and ecosystem functioning. These may be of a small or subtle nature. Therefore, risks arising out of cultivating

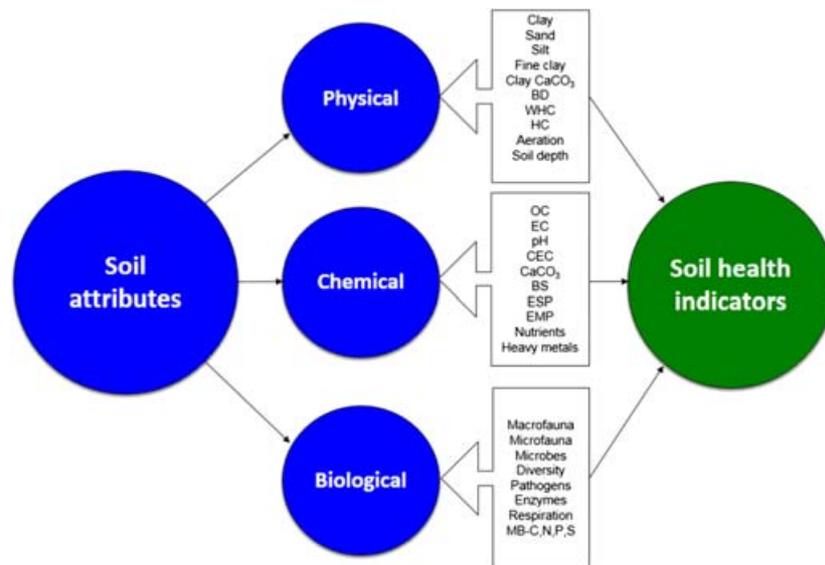


Figure 1. Some of the soil physical, chemical and biological attributes used as soil health indicators.

a transgenic crop need to be assessed. Another important aspect of the bio-safety assessment of transgenic crops is to study their impact on soil ecosystem including changes in the soil properties, soil associated micro-flora, micro- and macro-fauna. Transgenic crops are produced by an insertion of specific pieces of nucleic acids into the plant's DNA using recombinant DNA technology [17]. In most cases, the aim of developing a transgenic crop is to introduce a new trait into the crop plant, which does not occur naturally in the species. Traditional plant breeding has limitations to exchange of genetic material only between sexually compatible close relatives of a given plant [18]. Transgenic plants are developed to show herbicide tolerance, resistance to diseases and insects, and possessing superior agronomic properties such as better product quality.

Presently, major transgenic crops cultivated on a commercial scale across the world are soybean (*Glycine max*), maize (*Zea mays*), cotton (*Gossypium* sp.), canola (*Brassica* sp.), potato (*Solanum tuberosum*), sugar beet (*Beta vulgaris*), alfalfa (*Medicago sativa*), papaya (*Carica papaya*), squash (*Cucurbita* sp.), tomato (*Solanum lycopersicum*), poplar (*Populus* sp.) and sweet pepper (*Capsicum* sp.) [19, 20]. Global market value of biotech crops, in 2015, was to an extent of US\$15.3 billion of which 72% was in the developed world and the remaining 28% in the developing countries [16].

Why Study Transgenic Crops?

Most of the transgenic events primarily used cauliflower mosaic virus 35S RNA promoter to develop transgenic crops, which induces constitutive expression of transgenic proteins. The major reasons for the growing interest in the study of transgenic crops are (i) promoters used to develop transgenic crops may act as toxins towards specific groups

of organisms through root exudation [21], (ii) insertion of foreign genes into a new genome may lead to the development of unexpected phenotypes (pleiotropy), and altered plant chemistry and metabolism [22], and (iii) the risk of transfer of antibiotic resistance genes to soil microorganisms because of the use of marker genes (*nptII*, *Gent* and *Tet*) for antibiotic resistance in transgenic crops [23]. Apart from insect-resistant transgenic crops, recently, many crop species, such as sugar beet, maize, cotton and canola were genetically engineered to confer herbicide tolerance with an aim to improve pre- and post-emergence weed control by using herbicides (e.g. glyphosate, glufosinate-ammonium) without harming the cultivated crop. Glyphosate (Roundup®) is a broad-spectrum herbicide that kills most plants by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase in plants that is essential for the aromatic amino acid synthesis [24]. However, glyphosate was reported to affect rhizobacterial community structure of glyphosate-tolerant maize [25] and Roundup Ready soybean [26]. Recently, the World Health Organization issued a statement that glyphosate may be a possible carcinogen. Biotech crops were developed with an intention to minimize the use of toxic chemicals in agricultural production. However, there are issues being flagged about their adverse effects on soil ecology. As a result, scientists laid greater emphasis on research to better understand impact of transgenic plants, direct and indirect (Figure 2), on natural and agricultural ecosystems.

Need for Transgenic *Bacillus thuringiensis* (Bt) Cotton

Cotton is the most important commercial fibre crop grown in more than 80 countries under tropical and subtropical regions. Apart from fibre, cotton is an important source

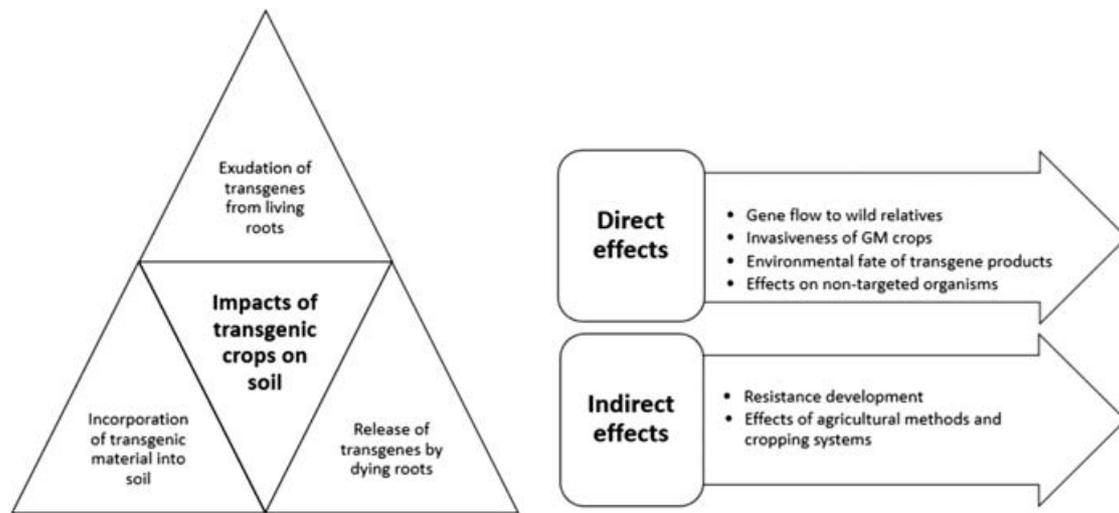


Figure 2. Direct and indirect effects of transgenic crops on soil.

of oil and high-quality protein. Since cotton is a long-duration crop, it is vulnerable to several insect pests including sucking pests, bollworms and defoliators at various stages of its growth. Among these pests, the cotton bollworm complex causes significant yield loss across the world. To control the bollworms, several new molecules of pesticides were introduced and their indiscriminate use has aggravated the problem of resistance build-up in insect pests, and consequently, leading to high cost of cotton cultivation [27]. This compelled a need for alternative technologies such as genetic modification to enable cotton plants to resist against bollworms. The age of *Bt* cotton began when Perlak *et al.* [28] introduced *cry1Ab* and *cry1Ac* genes from a soil bacterium *Bt* into cotton plants. The resulting transformants showed a high level of resistance to the cotton bollworm (*Helicoverpa armigera*). The *Bt* cotton was also active against other insects belonging to the orders: Lepidoptera, Diptera and Coleoptera [29]. Lately, gene stacking involving >1 gene has become an attractive alternative for an effective control of greater number of insect pests in cotton.

Presently, *Bt* cotton is cultivated in nine countries; namely, USA, Mexico, China, Argentina, South Africa, Colombia, India and Brazil [16]. Compared with the non-*Bt* (traditional cotton), *Bt* cotton cultivars not only gave higher yields but also higher net income because of reduced plant protection costs [30]. Although there is a large-scale adoption of *Bt* cotton by the farmers, there is a growing concern about the *Bt* toxin produced in leaves, stems and roots of *Bt* cotton plants getting introduced into the soil which may affect soil health [31–33]. Because of this growing interest among soil scientists, microbiologists, agronomists and environmentalists, various soil biological properties are being monitored following *Bt* cotton [34–40]. Some studies laid emphasis on the risk of growing *Bt* cotton on micro-flora and fauna in diverse agro-ecosystems [41–45]. While some studies indicate that

Bt cotton had no detrimental effects on soil flora and fauna [31, 46], *vice-versa* was reported by others [47, 48].

Impact of *Bt* Cotton on Soil Properties

Persistence of Cry proteins in soil

Bt is reported to be moderately persistent in soil having an average half-life of 4 months [49] but tends to increase and sporulate within specialized niches of pH and nutrients such as in insect cadavers. Studies by Donegan *et al.* [50] on the effect of the *Bt* toxin on bacteria and fungi was unsuccessful to show any influence on growth and species composition of soil microorganisms apart from a transitory increase in numbers. Vilas-Bôas *et al.* [51] showed that *Bt* vegetative cells did not multiply and sporulate in the soil microcosm, even in sterilized soil, and spore germination has never been demonstrated in non-sterile soil. The toxins from *Bt* subsp. *kurstaki* (active against Lepidoptera) and *Bt* subsp. *tenebrionis* (active against Coleoptera) are tightly bound to clay minerals [52, 53]. The cry toxin concentration was reported to be greater in the deep black soil as compared with shallow soil because of moisture differences [54]. A summary report on persistence in soil of *Bt* cotton cry toxin is presented in Table 1.

Bt cotton on soil function

Soil enzymes are one of the potential biological indicators of soil quality, because of their association to soil biology. Many soil enzymes including urease, dehydrogenase (DHA), phosphatase, glucosidase, aryl sulphatase, etc. participate in various biogeochemical cycles in soil. Hence, measurement of soil enzyme activities provides rapid response to changes in soil management. Several

Table 1 Persistence of Cry proteins in soil

Cry protein	Experimentation	Conclusions	References
Cry 1Ab Cry1Ac	Soil amendment with purified protein or <i>Bacillus thuringiensis</i> (<i>Bt</i>) cotton biomass	Purified protein was detected up to 28 days and the protein from <i>Bt</i> cotton was detected up to 56 days	[50]
Cry 1Ab Cry1Ac Cry3Aa	Soil amendment with <i>Bt</i> cotton biomass	No persistence of proteins in soil; proteins degraded in soil with a half-life of 20 days	[55]
Cry 1Ab Cry1Ac	Soil amendment with purified protein or <i>Bt</i> cotton biomass	Purified proteins and Cry proteins decreased rapidly, with a half-life of approximately 4 and 7 days	[56]
Cry1Ab	Soil amendment with purified protein	Protein still detectable in soil after 234 days by larvicidal assay	[57]
Cry 2A	Soil amendment with <i>Bt</i> cotton biomass <i>Bt</i> cotton cultivation	Half-life of bioactivity was estimated at 15.5 days by insect assay Half-life of bioactivity was estimated at 31.7 days by insect assay	[58]
Cry 1Ac	<i>Bt</i> cotton cultivation	No detectable level of protein in soil for 3–6 consecutive years	[59]

Table 2 Transgenic cotton on soil functions

Protein	Process/function	Experiment	Findings	References
Cry1Ac	Urease activity, nitrate reductase, acid and alkaline phosphatases	Soil with <i>Bacillus thuringiensis</i> (<i>Bt</i>) and non- <i>Bt</i> cotton	No significant difference enzyme activities	[33]
Cry1Ac	Selected enzymes	Soil amended with <i>Bt</i> and non- <i>Bt</i> cotton biomass	Biomass of <i>Bt</i> cotton stimulated the activities of all enzymes	[39]
Cry1Ac	N mineralization and Olsen-P	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	Total mineral-N was reduced in <i>Bt</i> cotton, whereas Olsen-P was increased	[40]
Cry1Ac	Root biomass	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	Root biomass were not different but root volume was significantly higher in <i>Bt</i> than non- <i>Bt</i> isoline	[40]
Cry1Ac	Microbial biomass C, N and P, organic carbon, microbial quotient, potential nitrogen mineralization, nitrification, nitrate reductase, phosphatase, root volume	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	No negative effects of <i>Bt</i> cotton on the indicators.	[46]
Cry1Ac	Soil respiration, fluorescein diacetate hydrolysis, urease, dehydrogenase, microbial biomass carbon	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	Higher biological activities in soil grown with <i>Bt</i> cotton than the non- <i>Bt</i> cotton	[61, 62]
Cry1Ac	Dehydrogenase and KMnO ₄ -N content	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	Positive correlations between <i>Bt</i> cotton cultivation and KMnO ₄ -N content and dehydrogenase in soil	[67]
Cry1Ac	Soil enzymes (urease, alkaline phosphatases, dehydrogenase, phenol oxidase, proteases)	Soil with <i>Bt</i> and non- <i>Bt</i> cotton	No differences in the activities of enzymes	[68]

authors studied changes in soil enzyme activities with relation to *Bt* cotton cultivation (Table 2). Higher urease activity was recorded by the addition of *Bt* cotton tissues in the soil [39, 60]. Mina *et al.* [33] reported higher urease, nitrate reductase, acid and alkaline phosphatase activities in *Bt* cotton cultivated soils. Similar results were supported by [46, 61, 62]. Soil DHA is considered as an indicator of the oxidative metabolism in soils and thus of the microbiological activity [63, 64] because it is linked to viable cells. DHA in soil depends on the content of soluble organic carbon [65] and the increased organic matter in the surface

soil horizon enhanced the soil enzyme activities. Studies by Furczak and Joniec [66] indicated that stimulation of DHA was accompanied by an increase in the number of the microbial groups and improvement in aeration and moisture. Positive correlations between DHA activity and *Bt* cotton cultivation was reported [61, 67]. Soil respiration rate was also unaffected by GM cotton [40, 46, 61, 62]. Substantial increase in Olsen-P by the cultivation of *Bt* cotton was reported [40]. Positive correlations between *Bt* cotton cultivation and KMnO₄-N content in soil have been reported [67].

Table 3 Cry proteins on soil invertebrates

Invertebrates	Cry protein	Experimentation	Conclusions	References
Micro-, meso- and macro-fauna	Cry1Ac	Cultivation of <i>Bt</i> and non- <i>Bt</i> cotton	Abundance of micro-, meso- and macro-fauna in <i>Bt</i> cotton rhizosphere	[33]
Collembola	Cry1Ab	Fed leaves of <i>Bt</i> and non- <i>Bt</i> cotton	No significant effects on oviposition, nos. of eggs and body length	[69]
Mites	Cry1Ac	Transgenic cotton	No adverse effect on earthworms	[70]
Earthworm	Cry1Ac	Cultivation of <i>Bt</i> and non- <i>Bt</i> cotton	No negative effects on numbers	[71]
Collembola	Cry1Ab			
	Cry1Ac			

Table 4 Cry proteins on soil microbial population and diversity

Microorganisms	Cry protein	Experimentation	Conclusions	References
Culturable bacteria and fungi	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	A significant, increase in numbers in soil with <i>Bt</i> cotton	[50]
Microbial population and diversity	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	Higher microbial population and diversity in <i>Bt</i> cotton	[61, 62]
Total microbial population	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	No adverse effects	[70]
Microbial functional diversity	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	No adverse effects	[68]
Culturable functional bacteria	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	No significant differences in numbers after the growing season	[74]
Methylobacteria	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	No adverse effects	[75]
Microbial diversity	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	No adverse effects	[76]
Culturable functional bacteria	Cry1A	Multiple-year cultivation of <i>Bt</i> cotton	No adverse effects	[77]
Composition of soil microbiota	Cry1Ac	<i>Bt</i> and non- <i>Bt</i> cotton	More extensive fungal colonization, higher ratios of fungi to bacteria, and different types of fungal spores in soil with <i>Bt</i> cotton	[86]

***Bt* cotton effects on soil micro-flora, micro- and macro-fauna**

Based on the available reports, *Bt* cotton showed no ill effects on soil invertebrates (Table 3). Based on their morphological features, oviposition, fecundity rate, etc., the soil invertebrates are not affected by growing *Bt* cotton.

Soil microbial population (soil and rhizosphere), their community structure (diversity) and associated microbial biomass is a potential indicator of changes in the soil as they are highly dynamic as compared with soil physical and chemical properties. Earlier studies inferred that qualitative and quantitative changes in root exudation of *Bt* cotton may possibly affect the structure of microbial communities, especially, in the rhizosphere [50, 72, 73]. Higher microbial counts in *Bt* cotton grown soil have also been reported by several workers [61, 62, 68, 74–76] (Table 4). Hu *et al.* [77] based on their multiple-year cultivation show that *Bt* cotton was not found to affect the rhizosphere functional bacterial population. Sarkar *et al.* [46] reported a significant correlation between root volume of *Bt* cotton and soil microbial biomass carbon that supports the findings of Lynch and Panting [78] that soil microbial biomass carbon (MBC) increased with root growth and rooting density of

the crop. Furthermore, significantly higher population of different microbial groups was reported in field plots under *Bt* cotton [50], alfalfa [79], papaya [80] and maize [81]. Saxena and Stotzky [31] did not observe any significant differences in the numbers of culturable bacteria, actinomycetes and fungi in the rhizosphere of *Bt* and non-*Bt* maize. Similarly, Brusetti *et al.* [82] detected no differences in the rhizosphere bacterial communities between *Bt* maize and its isogenic counterpart. However, some researchers observed minor to significant effects of cry proteins and *Bt* crops on soil microbial community structure. According to Petras and Casida [83], crystal proteins may serve as a substrate for the soil microbes. Therefore, an addition of *Bt* subsp. *kurstaki* to the soil results in a minor increase in populations of nematodes and micro-flora such as bacteria, actinomycetes and fungi. A significant but transient increase in the populations of culturable bacteria and fungi was observed in soil amended with *Bt* cotton leaves expressing *cry1Ac* protein [50]. The cry toxin had relatively less influence on the bacterial community structure than environmental factors, plant age or field heterogeneity [84]. However, Dunfield and Germida [85] observed that cultivation of *Bt* cotton leads to an undue decrease in microbial community richness.

Summary and Conclusions

Thus far, all available evidence shows that there have been no adverse or significant effects on soil health in terms of soil biology and ecology by growing *Bt* cotton. In general, the Cry proteins released in root exudates and from plant residues of *Bt* cotton had no consistent, significant and long-term effect on the soil micro-flora. Comparing soils from the *Bt* and non-*Bt* cotton fields, some differences were evident with regard to the microbial community structure and their population. However, most of these observations were of a transient nature. Furthermore, since a majority of these studies were not statistically significant, it cannot be inferred that the differences were due to the inserted *Bt* transgene. Using the classical and molecular techniques, however, few studies did report significant differences in microbial community structure in soils with *Bt* and non-*Bt* cotton. From the available literature, there is little evidence about crystal protein accumulation in soils, even after years of continuous *Bt* cotton cultivation.

Transgenic *Bt* cotton whether they pose any major risk to food and environmental safety should be put to rest by the national regulatory systems. These regulatory bodies should also instil confidence in the producers that there are no adverse economic impacts. More importantly, producers/farmers in the developing countries need to be educated about *Bt* cotton technology and remove any misconceptions about its adoption. Further, Government policy on seed pricing of transgenic crops is the need of the hour particularly in the developing countries, where seed cost contributes a major portion of the cultivation costs. Labelling must be mandatory for all the transgenic crops either edible or non-edible because of human health concerns.

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