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Detection and Quantification of *Bt* Toxin in Trannsgenic (*Bt*) Cotton Rhizospheric Soil of Northern India

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Abstract

The level of Cry1Ac protein in roots and soil planted with *Bt* cotton (Mech 162 + *Bt*) was evaluated during cropping season and after post-harvesting. Estimation of *Bt* toxin in the root samples viz. tap roots, fine roots revealed high concentration of *Bt* toxin (0.55 µg/g) was in tap roots followed by 0.25 µg/g in fine roots at late flowering stage (150 DAS). The concentration of the toxin in fine roots reached upto 0.34 µg/g at the maturity phase (165 DAS) and it remained almost stable upto 247 days. In the earlier stages of the crop (vegetative to flowering stage), we couldn't detect the *Bt* toxin in soil. However, in the late flowering stage (120 DAS), a concentration of 0.0012 µg/g Cry1AC toxin was detected in the soil which increased exponentially to 0.0033 µg/g in 195 DAS and then remained almost stable till 247 DAS. Our findings suggest that the Cry1Ac protein is still being passively released through the tap roots and fine roots in the standing crop even after the crop harvest, which was found to persist in the soil.

Key words : Cry1Ac toxin, Detection, Soil, Root, *Bt* cotton.

The year 2002 marked itself as the foundation year for commercialization of *Bt* cotton in India which was preceded by on farm field trials in 2001 on 395 farms in seven different states of India, where it showed reduction in pest damage and increased yield (1). Due to increased *Bt* cotton yield, exports of raw cotton from India soared from 0.9 million bales in 2005 to 4.7 million bales in 2006, the highest ever (2). GM agriculture can have the potentiality to decrease environmental pollution, increased foreign exchange through exports and ultimately net benefit to the farmers. Besides the measurement of tangible benefits, the intangible risks also need to be addressed thoroughly, as the speculated views about some above ground risks of GM agriculture have been proven in last few years by intensive research works throughout the world. (3). Amongst the other intangible risks, the impact of GM crops on the belowground ecological integrity need special concern as the toxins from *B. thuringiensis* introduced in transgenic plants and microbes could persist, accumulate, and remain insecticidal in soil as a result of binding on clays and humic substances and therefore pose a hazard to non-target organisms (4,5). Since different types of soil as well as climate interact differently in terms of degradation and persistency of *Bt* toxin in soil, research work should be done keeping the consideration of different geographical as well as temporal variation in mind. Till now no significant research in the field of underground speculated risk has been done in Indian context. Thus, the main objective of this study was to detect and determine the amount of *Bt* toxin released through roots under tropical conditions and the extent of its persistence in the soil.

Methods

The study was conducted at transgenic research farm of Indian Agricultural Research Institute (IARI), New Delhi (28°40′ N and 77°12′ E, at an altitude of 228 m above mean sea level). The soil of the experimental site was loamy (46% sand, 33% silt and 21% clay) with a bulk density of 1.38 g cm^3. The other properties of field soil were : pH (1 : 2 soil : water) 8.1, electrical conductivity 0.48 dS / m, CEC of 7.3 C mol (p +)/kg, organic carbon 4.5 g/kg, total N 0.30 g/kg, Olsen P 0.007 g/kg, and ammonium acetate extractable K contents 0.13 g/kg. Transgenic cotton seeds (MECH-162 + *Bt*) and its near isogenic line (MECH-162-*Bt*) from Maharashtra Hybrid Seeds Company Ltd., Mumbai were sown at 4-5 cm depth in second fortnight of June. All standard agronomic and crop management

practices were followed to ensure uniform germination and better standing of crop. Harvesting was done manually from early November to December and after the harvesting, the crop was left as such till February in the field to know the fate of the *Bt* toxin in the roots and in the soil. The experiment was repeated for two consecutive year's i.e.2006 and 2007. For quantification of *Bt* toxin (Cry 1Ac) in tap roots and fine roots of *Bt* cotton and its near isogenic line, the whole cotton plants were uprooted from four different *Bt* plots and its near isogenic line plots. Tap roots, fine roots were separated from the main root system of all the plants and stored at –20 C till further analysis. Soil samples from rhizospheric zone (0—15 cm)of *Bt* cotton and its near isogenic line plots was collected through out the cropping season from five different locations of the plot and were mixed to make them homogenous. Soil samples were stored at 20C till furteher analysis. There were three replications of each treatment and experiments were repeated at least twice. Quantification of *Bt* toxin in rhizospheric soil and roots (tap root and fine roots) of *Bt* cotton (MECH 162 +*Bt*) and its near isogenic line plants (MECH 162-*Bt)* was done by using Envirologix CrylAb/ Cry1Ac Quanti Plate kit given by Gupta and Watson (6). The processing and quantification of the toxin in roots was carried out as per the manufacturer's instructions, whereas the processing and quantification of the toxin in soil following the method of Baumgarte and Tebbe (7). The quantification of *Bt* toxin in the soil was confirmed in laboratory condition by decomposition study (soils were amended with 0.1, 0.5and 2% air dried *Bt* cotton (MECH 162 + *Bt*) leaves of *Bt* cotton leaves by alkali trap method.

Results and Discussion

Our study revealed that in the earlier stages of the crop (vegetative stage to flowering) we couldn't detect the *Bt* toxin in soil. However, in the late flowering stage i.e.at 120 days after sowing (DAS) of *Bt* crop 0.0012 µg/g of Cry1Ac toxin level was detected in the soil which increased exponentially to 0.0033µg/ g in 195 DAS and then remained almost stable till 247 DAS with Cry1Ac expression level of 0.0029 µg/g (Fig. 1). Although the crop was harvested from the field at 195 DAS, we continued the post harvest sampling for determining the extent of release of *Bt* toxin from roots

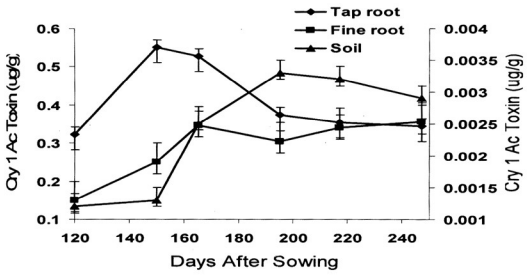

Figure 1. Temporal variation in CrylAc expression (µg ± SE) in roots and soil of *Bt* cotton.

and its persistence in the field. The presence of *Bt* toxin in soil is not suprising as the field and laboratory studies have demonstrated that *B. thuringiensis* toxin is released in soil through root exudates (8), post harvesting ploughing of plant residues (9), pollen deposition on the soil (3). In soil, toxin is known to bind tightly to surface active particles such as humic acids and clay minerals (10) which protect them from microbial degradation.

To quantify the *Bt* toxin in roots of *Bt* cotton, we started measurement of *Bt* toxin in roots from the flowering stage onwards, as it is already established that high amount of root exudates are released during flowering stage. Estimation of *Bt* toxin in the root samples (viz. lap roots, fine roots) revealed high concentration of Bt toxin 0.55 µg/g in tap roots and 0.25 µg/g in fine roots at late flowering stage (150 DAS). At 165 DAS, the concentration of the *Bt* toxin in tap roots remained almost stable, whereas it increased to 0.34 µg/g in fine roots. On further sampling of the roots in the harvesting stage i.e. 195 DAS, the concentration of CrylAc declined in tap roots by 28%. The concentration in the fine roots which increased earlier till 165 DAS did not show any decline rather it remained almost stable till 247 DAS. The level of *Bt* toxin in tap roots of Sicot 289i and Sicot 289RRi was estimated to be 4.9 µg/g and 3.5 µg/g respectively and in the fine roots it was 7.0 µg/g and 10.55µg/g at 65 DAS (7). Thus, the *Bt* cotton may release the Bt toxin into the soil either by the passive exudation or through root turnover or sloughed epidermal cells (6). However, the *Bt* toxin in root exudates of transgenic corn, potato and rice was even at larvicidal (*Helicoverpa armigera*) level (11). In our laboratory based decomposition study we could detect

Table 1. Amount of *Bt* toxin (CrylAc) in different time interval amended with *Bt* cotton leaves in soil.

	Treatments and toxin concentration (µg/g soil) (%)				
Days	Control	0.1	0.5	1	2
14 days	–	<1.2	3.01 ± 0.1	4.19 ± 0.02	4.29 ± 0.1
28 days	–	<1.2	2.5 ± 0.1	3.6 ± 0.01	3.8 ± 0.1
42 days	–	<1.2	1.9 ± .02	2.7 ± 0.022	2.9 ± 0.1
56 days	–	<1.2	<1.2	<1.2	<1.2

the toxin even 42 days after the experiment started (Table 1). This result support our previous result and reveals that transgenic cotton plants release the toxin in the soil and that possesses a tendency of persistence in soil even after a long time lapse.

To the best of our knowledge this is the first report from India that has indicated the presence of *Bt* toxin in the roots and in soil of the *Bt* cotton. Our findings of persistence of *Bt* toxin in soil up to 247 days are in consistence with the findings of Saxena and Stotzky (12) who reported that *Bt* toxin from transgenic corn biomass can persist in soil up to 350 days.

In view of the geographical difference and the high amount of intra-plant and in-season variability in CrylAc expression in *Bt* cotton plants, it would be difficult to compare the expression estimates because the CrylAc gene is in hemizygous form in the varieties grown in India as compared to homozygous form in other varieties grown in India as compared to homozygous form in other varieties grown in other parts of the world. The persistence of the toxin in the soil even after post harvesting, indicates that the released toxin has been protected from biodegradation in loamy soil. However, whether the mere presence of the toxin at this level in soil point towards the speculated changes in microbial diversity structure and key ecological functions remains to be explored. Moreover, for evaluating and assessment of the risk of transgenic on belowground ecosystem at the national scale, a better insight into the cause effect relationship in different agro-ecological scenarios is needed.

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