Impact of Transgenic Bt Cotton on Functional Fungal Populations in Rhizosphere Soil

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***Abstract*—**The environment release of transgenic plants may be accompanied by ecological effects and biosafety assessment. An important aspect of the biosafety assessment of genetically modified plant is to study its impact on soil ecosystem including changes in plant associated microflora. In the present investigation the effect of BT cotton plant expressing Bacillus thuringiensis (BT) cry1Ac on soil mycoflora was evaluated. BT cotton plant along with its non-BT counterpart was grown for BTaining various observations in the experimental farm of the university. Soil samples were collected at set time points from the rhizospere of the BT and non-BT cotton plants and were analyzed for the soil fungal population. Differences in the rhizosphere microflora between BT and its non-BT cotton counterpart were observed at different age of the plant.

***Keywords*—** BT and Non BT cotton, cry 1 Ac, Rhizosphere, soil mycoflora.

I. Introduction

IN India, the area under transgenic crop cultivation has witnessed a phenomenal growth, which is an increase of about 300 % in a year period [1]. Although there is large-scale adoption of Bt-cotton by the farmers because of immediate financial gain, there is concern that transgenic Bt-crops (which release Bt-toxins into the environment) affect nutrient cycling in the agroecosystem. This toxin is produced in every major part of Bt-cotton plants (leaves, stems and roots) [2]. During crop growth, soil micro-organisms come into direct contact with transgenic Cry endotoxin as it is released from Bt-crops in root exudates and from decomposing tissues [3]. Thus, the Bt-toxin has the potential to enter the soil system throughout the Bt-cotton-growing season, through root release and root turnover processes [3]. While Bt occurs naturally in soil, growth of transgenic Bt-crop causes a large increase in the amount of Cry endotoxin present in agricultural systems, e.g. roughly 0.25 g ha-1 produced naturally (calculated from approximately 1000 Bacillus thuringiensis spores g -1soil) vs. 650 g ha-1 in case of Bt-corn crop, excluding grain [4]. Thus the transgenic plants, either through the products of introduced genes and modified rhizosphere chemistry or through altered crop residue quality, have the potential to significantly change the essential ecosystem functions such as nutrient mineralization, carbon turnover and plant growth [3], [5], [6].

Cry proteins from transgenic cotton may be introduced into the soil through rhizosphere exudates [7] or from decomposing crop residues. Cry proteins could affect the rhizosphere or soil microbial communities directly or indirectly. BT corn was reported to have no effects on numbers of culturable microorganisms [8], while transient increases in culturable microorganisms were observed with BT cotton [9] and BT potato [10]. BT corn had no impact on microbial biomass, activity, or community structure [11]. We are investigating the impact of BT cotton expressing different Cry proteins, on rhizosphere and soil microbial communities.

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II. Materials And Methods Soil Sampling

Soil samples where collected from the rhizosphere of BN-BT cotton and its non-BT counterpart grown at the CICR farm at 30, 60 and 90 days after sowing. Area of 1ft around the plant was marked and represented as the rhizosphere soil. Each rhizosphere soil sample was comprised of 5 core samples drawn from 5-6 inches deep and 3 inch wide bore made around the rhizosphere of 5 randomly selected BT plant. The soil was also drawn by the same procedure from the rhizosphere of non-BT plants too. The soil from the two treatments was mixed thoroughly in separated containers. From this 0.5 kg soil was drawn as representative rhizosphere sample.

Determination Of Total Fungal Population

To determine the total fungal populations, rhizosphere soil were homogenized in a pestle to break the clods. Samples were prepared in sterile distilled water by suspending 1 gm soil in 100 ml water and shaking the sample vigorously for 20 min on an orbital shaker at 250 rpm. The primary soil suspensions were serially diluted further and 10-4 dilutions were plated on Rose-Bengal supplemented potato dextrose agar (PDA) media in Petri plates to determine populations of fungal mycoflora. The plates were incubated at 27-30oC for 7 days and observed for the appearance of colonies. The population count of the organisms was recorded after the stipulated incubation periods. The differences in the fungal populations in BT and Non-BT cotton rhizosphere were determined.

III. Results And Discussion

In order to understand the change in fungal population at different age of the crop the analysis of fungal population was made from different periods like 30, 60 and 90 days age of the crop, in case of both BT and non-BT isolines. Results are summarized in table 1 to 3. Table 1,2 and 3 presented the eighteen fungi viz. Alternaria alternata, Alt. solani, Aspergillus flavus, Asper. Niger, Cladosporium sp., Curvularia lunata, fusarium moniliformae, Fusarium oxysporium, Fus. Roseum, Penicillium notatum, Phoma exigua, Phytopthora sp., Nigrospora oryzae, Rhizoctonia solani, Rhi. Bataticoli, Trichoderma harzianum, Trichoderma viride were found to be associated with the rhizosphere of both BT and Non-BT varieties to a more or less extent.

In the initial attempts analysis of rhizosphere fungal population was carried out at different age of the crop using BT cotton varieties and their non-BT cotton isolines growing in fields. The results clearly indicate that fungal population in the rhizosphere gradually increases with the age of the crop and it was at the climax upto 60 days age of the crop. Then with increase in age of the crop fungal population declined. This clearly suggests that biochemicals produced in the plant they might be changing qualitatively in the plants and therefore that is being responsible to affect rhizosphere fungal population either growing maximum when the crop is fully matured and with rich vegetative growth or the fungal population declined when the crop was on the verge of senescent stage.

Similar type of reports regarding changes in rhizosphere mycoflora with different age of crop have been recorded earlier by [12] in case of cotton.

The evaluation of rhizospheric fungi was found to be differing within BT cotton varieties and their isolines non-BT cotton varieties qualitatively and quantitatively. Even every variety of BT cotton and non-BT cotton isolines show slight difference. The total fungal population was clearly higher in non BT cotton isolines as compared to that of all three BT cotton varieties. It seems that the biochemicals or exudates released by BT cotton varieties may have some impact on the occurance of fungi and hence the qualitative evaluation clearly shows poor population in BT cotton varieties.

Similar results like Cry protein is known to persist longer in clay soils [7] were found. This toxin is produced in every major part of Bt-cotton plants (leaves, stems and roots) [2]. During crop growth, soil micro-organisms come into direct contact with transgenic Cry endotoxin as it is released from Bt-crops in root exudates and from decomposing tissues [3]. Thus, the Bt-toxin has the potential to enter the soil system throughout the Bt-cotton-growing season, through root release and root turnover processes [3]. This results conclude at a point in such a way that toxin secreted by BT cotton plant through root exudates have effect on soil mycoflora. Affected fungi are both useful and harmful. It's good that if root exudates shows effect on harmful fungi but what about useful fungi.

References

[1] James, C. 2006: Global Status of Commercialized Biotech/GM Crops: 2006. ISAAA Brief No. 35. ISAAA, Ithaca, NY.

[2] Dong, H. Z., and W. J. Li, 2007: Variability of endotoxin expression in Bt transgenic cotton. J. Agron. Crop Sci. 193, 21–29.

[3] Motavalli, P. P., R. J. Kremer, M. Fang, and N. E. Means, 2004: Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J. Environ. Qual. 33, 816–824.

[4] Blackwood,C. B., and J. S. Buyer, 2004: Soil microbial communities associated with Bt and non-Bt corn in three soils. J. Environ. Qual. 33, 832–836.

[5] Dunfield, K. E., and J. J. Germida, 2004: Impact of genetically modified crops on soil- and plant-associated microbial communities. J. Environ. Qual. 33, 806–815.

[6] O'Callaghan, M., T. R. Glare, E. P. J. Burgess, and L. A. Malone, 2005: Effects of plants genetically modified for insect resistance on nontarget organisms. Annu. Rev. Entomol. 50, 271–292.

[7] Saxena, D., S. Flores and G. Stotzky. 2002. BT toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biol. Biochem. 34:133-137.

[8] Saxena,D. and G. Stotzky. 2001. Bacillus thuringiensis (BT) toxin released from root exudates and biomass of BT corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil. Soil Biol. Biochem. 33:1225-1230.

[9] Donegan, K. K., C. J. Palm,V. J. Fieland, L. A. Porteous, L. M. Ganio, D. L. Schaller, L. Q. Bucao and R. J. Seidler. 1995. Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing Bacillus thuringiensis var. kurstaki endotoxin.Appl. Soil Ecol. 2:111-124.

[10] Donegan, K. K., D. L. Schaller, J. K. Stone, L. M. Ganio, G. Reed, P. B. Hamm and R. J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the Bacillus thuringiensis var. tenebrionis endotoxin. Transgenic Res. 5:22-35.

[11] Devare, M. H., C. M. Jones and J. E.Thies. 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. J. Environ. Qual. 33:837-843.

[12] Iyer, V.N. 1961. Micororganisms associated with the rhizosphere of the O cotton plant. Proc. Indian Acad. Sci. LIV (B) : 167-169.

TABLE I

FUNGAL POPULATION ($X10^{-4}$) OBSERVED IN RHIZOSPHERE OF BT AND NON BT. COTTON VARIETIES AFTER 30 DAYS OF SOWING

0	BT-cotton varieties			Non BT-cotton varieties		
	Variety-1	Variety-2	Variety-3	Variety-1	Variety-2	Variety-3
Rhizopus oryzae	0.33	0.33	1.66	0.33	1.00	0.66
Aspergillus niger	2.33	2.00	0.66	2.00	2.66	2.00
Aspergillus flavus	1.11	3.11	1.33	3.11	1.66	0.66
Phoma exigua	0.00	0.00	0.00	0.00	0.33	1.66
Nigrospora oryzae	0.00	0.66	0.00	0.66	1.00	0.33
Penicillium notatum	0.66	0.33	1.33	0.33	0.66	1.33
Trichoderma viride	2.33	0.66	0.00	0.66	1.00	0.00
Trichoderma harzianum	0.00	0.00	0.00	0.00	1.00	1.66
Fusarium oxysporum	2.66	0.66	2.00	0.66	0.66	2.33
Fusarium moniliformae	0.33	0.66	0.66	0.66	1.33	0.33
Cladosporium spp.	0.33	0.66	0.00	0.66	1.00	0.33
Fusarium roseum	2.00	1.00	1.00	1.00	0.66	1.00
Curvularia lunata	0.33	0.33	0.33	1.33	0.66	0.33
Alternaria alternata	0.33	0.66	0.66	0.66	1.00	0.33
Alternaria solani	0.66	1.66	1.66	1.66	1.00	1.33
Rhizoctonia solani	0.66	1.00	1.33	1.00	0.66	0.33
Rhizoctonia bataticoli	1.66	0.33	1.33	0.33	0.33	0.66
Phytophthora spp.	0.00	0.00	0.00	0.00	1.33	1.00

TABLE II

FUNGAL POPULATION ($X10^{-4}$) OBSERVED IN RHIZOSPHERE OF BT AND NON BT. COTTON VARIETIES AFTER 60 DAYS OF SOWING

Fungi isolated	BT-Cotton varieties			Non BT-Cotton varieties		
	Variety-1	Variety-2	Variety-3	Variety-1	Variety-2	Variety-3
Rhizopus oryzae	0.00	1.33	0.33	0.00	0.00	0.00
Aspergillus niger	1.33	1.00	1.66	2.00	1.66	1.33
Aspergillus flavus	1.00	0.66	1.33	1.33	1.33	1.00
Phoma exigua	0.00	0.00	0.00	0.00	0.66	2.00
Nigrospora oryzae	0.00	0.00	0.00	0.00	1.33	1.33
Penicillium notatum	0.66	1.00	1.66	3.11	2.00	1.00
Trichoderma viride	1.33	0.00	1.33	1.33	1.00	1.00
Trichoderma harzianum	0.00	0.00	0.00	0.00	0.66	0.66
Fusarium oxysporum	0.00	1.66	0.00	0.00	0.33	0.33
Fusarium moniliformae	0.00	0.66	0.33	0.00	0.00	0.33
Cladosporium spp.	0.00	0.00	0.00	0.00	0.66	1.33
Fusarium roseum	0.00	0.33	0.00	0.00	1.00	0.66
Curvularia lunata	0.66	0.66	0.00	0.66	0.00	0.33
Alternaria alternata	0.00	0.66	0.33	0.00	0.00	0.00
Alternaria solani	1.33	0.00	2.66	2.00	1.66	1.66
Rhizoctonia solani	0.00	0.66	0.00	0.00	0.66	1.33
Rhizoctonia bataticoli	1.66	0.00	1.33	1.66	1.66	0.66
Phytophthora spp.	0.00	0.00	0.00	0.00	1.33	0.66

TABLE III

FUNGAL POPULATION (X10-4) OBSERVED IN RHIZOSPHERE OF BT AND NON BT. COTTON VARIETIES AFTER 90 DAYS OF SOWING

Fungi isolated	BT-Cotton varieties			Non BT-Cotton varieties		
	Variety -1	Variety-2	Variety-3	Variety-1	Variety-2	Variety-3
Rhizopus oryzae	0.00	0.66	0.00	0.66	0.00	0.33
Aspergillus niger	2.00	3.00	1.33	1.00	3.00	2.33
Aspergillus flavus	1.33	1.00	1.00	0.33	1.00	1.33
Phoma exigua	0.33	0.00	2.00	0.66	1.00	0.00
Nigrospora oryzae	1.00	0.00	0.33	1.00	1.00	0.00
Penicillium notatum	1.33	2.00	1.00	1.33	1.33	0.33
Trichoderma viride	2.66	0.33	1.66	0.00	1.66	1.00
Trichoderma harzianum	0.00	0.00	0.00	0.00	1.33	2.00
Fusarium oxysporum	0.00	1.66	0.00	1.66	0.00	0.66
Fusarium moniliformae	0.00	0.66	0.33	1.33	0.00	0.66
Cladosporium spp.	0.00	0.00	0.33	0.00	0.33	1.33
Fusarium roseum	0.00	0.33	0.00	0.00	0.66	0.33
Curvularia lunata	0.00	1.00	0.33	1.00	1.66	1.00
Alternaria alternata	0.00	0.66	0.33	1.00	0.33	1.33
Alternaria solani	1.66	0.00	1.66	0.00	0.33	1.33
Rhizoctonia solani	0.33	1.33	0.00	2.33	0.00	0.33
Rhizoctonia bataticoli	0.00	0.33	1.00	1.00	0.33	1.00
Phytophthora spp.	0.33	0.00	0.33	0.66	0.66	0.33