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Effect of Bt cotton on nutrient dynamics under varied soil type

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Abstract

Since transgenic cotton was first grown commercially in India in 1996, the areas cultivated have increased rapidly around the world. Bt cotton is produced by inserting a synthetic version of a gene from the naturally occurring soil bacterium *Bacillus thuringiensis* into cotton. Bt cotton may affect nutrient dynamics in many ways during its life-span with regard to the temporal-spatial relevance of Bt proteins. Given this, we aimed to evaluate nutrient availability under both Bt and non-Bt systems and varied soil type. The study was conducted during the 2010 wet season (July to December) in a net-house at the Institute of Agricultural Sciences of Banaras Hindu University. It was carried out on three different soil orders *i.e.* entisol, inceptisol and alfisol. Bt cotton (*cv*NCS-138) and its non-transgenic isoline (cvNCS-138) were grown until maturity. A *no crop* pot was maintained with three replications for all the three soil orders. Study design was a factorial experiment under a completely randomized block design with three replications. The study concludes that available N was reduced by 12-13% under Bt cotton compared to non-Bt isoline and *no crop* treatment whereas it showed a significant increase in available P in the soil under Bt cotton (7.8% increase) compared to non-Bt isoline and *no crop* treatment. Furthermore, it has been observed that available K value varied from 82.88 kg ha^{-1} to 76.88 kg ha^{-1} in the soil under Bt cotton and from 90.33 kg hato^{-1} 83.55 kg ha^{-1} in the non-Bt crops and a significant increase in the Avalaible Zn in the soil under Bt cotton compared to non-Bt isoline and *no crop* treatment.

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Introduction

Cotton (*Gossypium hirsutum L.*) is the most important fiber crop in India and plays a key role in economic and social affairs. Its cultivation, processing and trade supports millions of people and contributes 360 billion Rupees to income from exports around the world. India has the largest area under cotton in the world (representing 20-25% of the global area) but it ranks only third in terms of production after China and the USA. Several factors are responsible for such low yields of which losses due to insect pests are the most important. More than 160 species of insects have been reported to attack cotton at various stages of its growth as defoliators, tissue borers and sap-suckers, causing losses of up to 60% (Qaim and Zilberman, 2003).

Genetic modification of organisms (plants, microbes and animals) to incorporate useful traits is powerful technology for the future development of sustainable agricultural systems. Transgenic cotton varieties modified to express the Cry1Ac insecticidal toxin (Bt cotton) that is toxic to some insect pests is now grown worldwide. Through i) the products of introduced genes, ii) modified rhizosphere chemistry, or iii) altered crop residue quality, genetically modified plants have the potential to significantly change microbial dynamics, soil biodiversity and essential ecosystem functions, such as nutrient mineralization, disease incidence, carbon turnover and plant growth. Wang *et al.* (2006) reported that the Cry1Ab protein from biomass of Bt rice degraded faster in alkaline soil (half-life 11.5 d) than in acidic soil (half-life 34.3 d). However, there are few experimental data available, especially quantitative data, on the environmental consequences of sustained expression and/or presence of Bt toxin in various parts of Bt cotton plants. In addition, very little is known about the potential for the persistence of Bt toxin released from Bt cotton plants in cotton soils (Sahai, 2003). Given this, our research was designed to evaluate nutrient dynamics under both Bt and non-Bt systems and varied soil type.

Materials and Methods

The study was conducted during the 2010 wet season (July to December) in a net-house on three different soil types at the Institute of Agricultural sciences, Banaras Hindu University, Varanasi (25° 19' 60 N Latitude and 83° 0' 0 E Longitude). Bt cotton (cvNCS-138) and its non-transgenic isoline (cvNCS-138) were grown until maturity. A *no crop* pot was maintained with three replications for all the three soil types.

Analysis of soil

The cultivated soils of three orders *i.e.* entisol, inceptisol and alfisol were collected from different geographical locations in the Varanasi

and Mirjapur districts of Uttar Pradesh, India. The soil used in the pot experiment was air dried, ground and sieved through a 2 mm sieve, and then stored in plastic bags. An initial soil sample was analyzed for pH in 1:2 soil water suspension using a combined electrode in a digital pH meter. Electrical conductivity was measured in the supernatant liquid of the soil water suspension (1:2) with the help of a conductivity bridge and expressed in dS m^{-1} at 25°C. Mechanical composition of the soil was determined by the hydrometer method (Bouyoucos, 1962). Water holding capacity was determined by methods outlined by Topp *et al.* (1993). Soil organic carbon content was determined by the wet oxidation method of Walkley and Black (1934). The available N was determined by an alkaline potassium permanganate method (Subbiah and Asija, 1956). Soil samples were extracted with a combination of HCl and NH4F (Bray and Kurtz, 1945) and 0.5 M $NaHCO_3$ (Olsen *et al.*, 1954) for available phosphorus. The ammonium acetate (pH 7) method was used to calculate CEC and available potassium of soils (Jackson, 1973). An estimate of the potassium in extract was made by flame photometry. The DTPA-extractable Zn was determined according to the procedure outlined by Lindsay and Norvell (1978) using an atomic absorption spectrophotometer.

Rhizosphere soil samples were analyzed for mineral-N, available P, available-K, and available Zn and these were estimated at regular time points throughout the growth stages of the plant. This was a factorial experiment under a completely randomized block design with three replications.

Results and Discussion

The chemical properties and texture of the soils before starting the experiment are shown in Table 1. Of the three soils, two were slightly alkaline and one was acidic in reaction. All the soils had low organic matter content. Soil bulk density varied from 1.38 to 1.51 Mg m-3, EC varied from 0.32 to 0.61 dSm^{-1}, CEC from 18.25 to 31.85 Cmol (p+) kg^{-1}, available N from 167 to 238 Kg ha^{-1}, P from 8 to 18 Kg ha^{-1}, K from 110 to 165 Kg ha^{-1} and Zn from 2.86 to 3.84 ppm, respectively.

Little information is available regarding the effects of transgenic crops on the availability of soil nutrients. Transgenic Bt-crops may affect nutrient cycling, i.e. nutrient mineralization in the agroecosystem, either through the products of introduced genes or modified rhizosphere chemistry. Available N was reduced by 12-13% under Bt cotton over non-Bt isoline and *no crop* treatment (Table 2). There may be a possibility of higher N uptake by Bt cotton when compared with the non-Bt isoline. The availability of mineral N in the soil at a particular time during crop growth may be affected by many factors, including crop growth itself. The nutrient demand of cotton is the highest at the boll formation stage (Gerik *et al.*, 1994). Transgenic plants may affect soil nutrient transformations (Motavalli *et al.*, 2004), but whether root exudates or other non-targeted physiological changes (*e.g.* content of starch, soluble N, proteins, carbohydrates, lignin) in the plant are responsible is still unclear (Icoz and Stotzky, 2008). Nitrogen immobilization by soil organisms during the decomposition of fine dead root biomass under Bt cotton may be a reason for the reduced available N content in the soil. Reduced dehydrogenase activity, and soil respiration rate and its strong association with mineral N, also indicate the possibility of reduced N mineralization in the rhizosphere of Bt cotton. The effect of the interaction between soil type and Bt-crop was found to be significant at different growth stages throughout the growing season. Soil samples were also analyzed for available P. The data (Table 3) revealed a significant increase in available P in the soil under Bt cotton compared to non-Bt isoline and *no crop* treatment. The available P content decreased throughout the growing stages. The increasing root length value and root biomass, as observed in the study, results in greater root exudates and readily metabolizable C and N. These are perhaps the most influential factors contributing to as much as a 7.8% increase in available P under Bt cotton. Among three different soils, alluvial soil recorded the highest available P content followed by black soil and red soil, probably because of variation in rhizosphere soil pH. Phosphorus availability in soil is generally influenced at the main interaction zone between the plant and soil biota near the root surface in the rhizosphere (Kennedy, 1998). Both plant roots and soil microorganisms alter and are affected by soil chemical and physical properties in the rhizosphere. Among the examples of factors affecting soil P

Table 1. Physical and chemical properties of initial experimental soil.

Parameters	Value		
	Red soil (alfisol)	Black soil (entisol)	Alluvial soil (inceptisol)
Physical			
Bulk density (Mg m^{-3})	1.38	1.51	1.43
Particle density (Mg m^{-3})	2.51	2.60	2.56
Water holding capacity (%)	39.4	45.40	41.6
Sand (%)	46.00	11.7	48.78
Silt (%)	32.85	52.7	30.48
Clay (%)	21.15	35.6	20.44
Soil texture	Silty clay loam	Clayey	Sandy loam
Electro-chemical and chemical properties			
pHw (1:2.5)	6.3	7.5	7.1
Electrical conductivity (dSm^{-1})	0.32	0.61	0.45
CEC [Cmol (p+) kg^{-1}]	18.25	31.85	19.55
Organic carbon (%)	0.34	0.42	0.38
Available nitrogen (kg ha^{-1})	176	238	232
Available phosphorus (kg ha^{-1})	8	14	18
Available potassium (kg ha^{-1})	110	165	148
Zn (ppm)	3.64	2.7	2.86

Table 2. Available N(kg ha^{-1}) in cotton rhizosphere soil at different growth stages.

DAS	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	155	211.33	217.33	194.55
	Bt (V_2)	150	208.66	202.66	187.10
	No crop (V_3)	105.4	143.70	130.4	126.50
	Mean	136.8	187.88	183.46	
	LSD (0.05)	C =1.49, S =1.49, CxS =2.59			
	SEm±	C =0.504, S=0.504, CxS=0.875			
100	Non-Bt (V_1)	146	203	204.66	184.55
	Bt (V2)	133.66	191.33	194	172.99
	No crop (V_3)	99.28	131.95	122.8	118.01
	Mean	126.31	175.42	173.82	
	LSD (0.05)	C =1.753, S=1.723, CxS=3.039			
	SEm±	C=0.584, S=0.584, CxS=1.013			
150	Non-Bt (V_1)	143.32	202.33	163.62	169.75
	Bt (V_2)	131	188.75	183.66	167.80
	No crop (V_3)	97.45	131.51	118.17	115.71
	Mean	123.92	174.19	155.15	
	LSD (0.05)	C = 1.75, S=1.75, CxS=3.03			
	SEm±	C= 0.583, S=0.583, CxS=1.011			

S_1, red soil; S_2 , black soil; S_3, alluvial soil; V_1, non-Bt cultivar; V_2, Bt cultivar; V_3, *no crop*; DAS, days after sowing.

availability, root exudates, such as organic acids, H+ ions, sugars and phosphatases, facilitate the solubilization and desorption of mineral P (Ryan *et al.*, 2001). Exogenous application of organic acids to soils and organic acid exudation from plant roots have been shown to improve the availability of P (Koyama *et al.*, 2000; Lopez-Bucio *et al.*, 2000). Alterations in the composition and quantity of root exudates through the introduction of new genetic traits may, therefore, directly affect processes, such as mineral P or fixed P solubilization, or indirectly affect the availability of P through changes in the activity of rhizosphere micro-organisms. It can, therefore, be presumed that P availability was enhanced because of changes in rhizospheric conditions under Bt cotton. This is consistent with increased mineral availability in the rhizosphere of transgenic alfalfa having increased exudation of citrate, oxalate, malate, succinate and acetate (Tesfaye *et al.*, 2001). A negative linear relationship of the available P with that of its root parameters was observed in the study, indicating that enhanced availability of P might not only be due to variation in root exudates, but perhaps also due to rhizospheric micro-organisms. The interaction effect between soil type and Bt-crop was found to be significant at different growth stages throughout the growing season.

Available K value varied from 82.88 kg ha^{-1} to 76.88 kg ha^{-1} in the soil under Bt cotton and from 90.33 kg ha^{-1} to 83.55 kg ha^{-1} in the non-Bt crops (Table 4). Changes in different values obtained in the soil were presumably due to variable clay mineralogy, clay content, or gravimetric moisture content. There was no significant difference in interaction between soil and variety factors.

Available Zn content in soil was determined throughout the growing

Table 3. Available P (kg ha^{-1}) in cotton rhizosphere soil at different growth stages.

DAS	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	8.21	10.75	21.73	13.56
	Bt (V_2)	10.55	12.40	24.66	15.87
	No crop (V_3)	5.58	6.98	13.06	5.200
	Mean	8.11	10.04	19.81	
	LSD (0.05)	C =0.434, S=0.434, CxS=0.752			
	SEm±	C =0.144, S=0.144, CxS=0.25			
100	Non-Bt (V_1)	7.63	9.25	13.7	10.19
	Bt (V_2)	9.66	11.32	19.82	13.59
	No crop (V_3)	5.19	6.01	8.22	6.470
	Mean	7.49	8.86	13.91	
	LSD (0.05)	C =0.314, S=0.314, CxS=0.544			
	SEm±	C=0.104, S=0.104, CxS=0.181			
150	Non-Bt (V_1)	7.18	8.50	12.43	9.37
	Bt (V_2)	8.42	10.61	18.67	12.56
	No crop (V_3)	4.88	5.52	7.46	5.95
	Mean	6.82	8.21	12.85	
	LSD (0.05)	C =0.314, S=0.314, CxS=0.544			
	SEm±	C=0.104, S=0.104, CxS=0.181			

S_1, red soil; S_2 , black soil; S_3, alluvial soil; V_1, non-Bt cultivar; V_2, Bt cultivar; V_3, *no crop*; DAS, days after sowing.

Table 4. Available K (kg ha^{-1}) in cotton rhizosphere soil at different growth stages.

DAS	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	99	86	86	90.33
	Bt (V_2)	89.33	79	80.33	82.88
	No crop (V_3)	67.31	55.9	51.2	58.13
	Mean	85.21	73.63	72.51	
	LSD (0.05)	C =2.03, S =2.03, CxS =1.92			
	SEm±	C =0.75, S=0.75, CxS=0.0.614			
100	Non-Bt (V_1)	87.6	83.66	82	84.42
	Bt (V_2)	82	79.33	75.66	78.99
	No crop (V_3)	59.61	54.38	49.2	54.39
	Mean	76.40	72.45	68.95	
	LSD (0.05)	C =2.366, S=2.366, CxS=1.664			
	SEm±	C=0.815, S=0.815, CxS=0.538			
150	Non-Bt (V_1)	87.66	83	80	83.55
	Bt (V_2)	80	76.66	74	76.88
	No crop (V_3)	59.61	53.95	48	53.85
	Mean	75.75	71.20	67.33	
	LSD (0.05)	C =2.39, S=2.39, CxS=1.95			
	SEm±	C=0.76, S=0.76, CxS=0.621			

S_1, red soil; S_2 , black soil; S_3, alluvial soil; V_1, non-Bt cultivar; V_2, Bt cultivar; V_3, *no crop*; DAS, days after sowing.

Table 5. Available Zn (ppm) in cotton rhizosphere soil at different growth stages.

DAS	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	3.28	3.49	2.43	3.06
	Bt (V_2)	4.32	3.49	4.54	4.11
	No crop (V_3)	2.22	2.26	1.52	2.00
	Mean	3.27	3.08	2.83	
	LSD (0.05)	C =0.488, S =0.488, CxS =0.923			
	SEm±	C =0.154, S=0.154, CxS=0.381			
100	Non-Bt (V1)	2.92	5.29	2.55	3.58
	Bt (V_2)	6.17	6.25	3.49	5.30
	No crop (V_3)	1.98	4.11	1.53	2.54
	Mean	3.69	5.21	2.52	
	LSD (0.05)	C =0.398, S=0.398, CxS=0.572			
	SEm±	C=0.126, S=0.126, CxS=0.0.181			
150	Non-Bt (V1)	2.61	4.54	5.61	4.25
	Bt (V2)	4.43	4.68	8.31	5.80
	No crop (V3)	1.77	2.95	3.36	2.69
	Mean	2.93	4.05	5.76	
	LSD(0.05)	C = 0.366 , S=0.366, CxS=0.517			
	SEm±	C= 0.116, S=0.116, CxS=0.164			

S_1, red soil; S_2 , black soil; S_3, alluvial soil; V_1, non-Bt cultivar; V_2, Bt cultivar; V_3, *no crop*; DAS, days after sowing.

Table 6. Soil organic carbon (g kg-1) of cotton at different growth stages.

DAS	Cultivar (C)	Soil types(S)			Mean
		S_1	S_2	S_3	
50	Non-Bt (V_1)	3.1	4.0	3.6	3.56
	Bt (V_2)	3.2	4.1	3.9	3.73
	No crop (V_3)	2.1	2.6	2.13	2.27
	Mean	2.8	3.56	3.21	
	LSD (0.05)	C =0.09, S =0.09, CxS =0.19			
	SEm±	C =0.0.041, S=0.041, CxS=0.0.090			
100	Non-Bt (V_1)	3.0	0.41	3.4	3.50
	Bt (V_2)	3.1	0.42	3.8	3.70
	No crop (V_3)	2.04	2.66	1.98	2.22
	Mean	2.71	3.65	3.06	
	LSD (0.05)	C =0.11, S=0.11, CxS=0.2			
	SEm±	C=0.054, S=0.054, CxS=0.093			
150	Non-Bt (V_1)	3.2	4.16	3.5	3.62
	Bt (V_2)	3.26	4.47	3.93	3.88
	No crop (V_3)	2.13	2.73	2.1	2.32
	Mean	2.86	3.78	3.17	
	LSD (0.05)	C = 0.081, S=0.081, CxS=0.0.175			
	SEm±	C= 0. 0.038, S=0.038, CxS=0.082			

S_1, red soil; S_2 , black soil; S_3, alluvial soil; V_1, non-Bt cultivar; V_2, Bt cultivar; V_3, *no crop*; DAS, days after sowing.

season. There was no significant difference in available Zn of cotton plants between the two cultivars (Bt and non-Bt) during their initial growth and final stage of harvesting for the three different soils, *i.e.* 50 DAS and 100 and 150 DAS, whereas the interaction between soil and variety factors are significant at 100 and 150 DAS but not at 50 DAS.

There was a significant increase in the available Zn in the soil under Bt cotton over non-Bt isoline and *no crop* treatment (Table 5). Higher root biomass mediated exudation and this was perhaps the most important reason for the increase.

The increasing root length value and root biomass observed in the study resulted in greater root exudates and readily metabolizable C. Of the three different soils, alluvial soil recorded higher soil organic carbon content (Table 6), followed by black soil and red soil, whereas a higher value of soil organic carbon in Bt cotton crop compared to non-Bt isoline was shown. This was probably because of changes in rhizosphere due to chemical composition of root or microbial properties in the rhizosphere of Bt cotton. There was no significant difference in the interaction effect between soil type and Bt-crop at different growth stages throughout the growing season. Plant residues are the primary source of metabolic energy (carbon) in soils and most biota populations and biota-mediated processes are concentrated in the rhizosphere and near crop residues.

Conclusions

Results showed that there were few significant differences in essential ecosystem functions such as nutrient mineralization with reference to nitrogen and potassium between Bt and non-Bt cottons at any of the growth stages and after harvest. All results suggested that there was no evidence to indicate any adverse effects of Bt cotton on the soil ecosystem in this study.

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