MINERAL DEFICIENCY STRESS

Transgenic *Bt*-Cotton Affects Enzyme Activity and Nutrient Availability in a Sub-Tropical Inceptisol

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Abstract

We investigated the dynamics of N and P availability in the rhizosphere of *Bt* and non-*Bt* cotton crops during their growth. In a net-house pot culture experiment at the Indian Agricultural Research Institute, New Delhi, *Bt*-cotton (cv. MRC-6301*Bt*) and its non-transgenic near-isoline (MRC-6301) were grown on a sandy loam soil until maturity. A control (no-crop) treatment was also included. Rhizosphere soil and root samples were collected at 60, 90, and 120 days after sowing (DAS). Soil samples were analysed for dehydrogenase activity, soil respiration, mineral-N and Olsen-P. Results have revealed a significant reduction in dehydrogenase activity (−17 %) and soil respiration (−3.5 %) in the rhizosphere of *Bt*-cotton over non-*Bt* isoline. Total mineral-N (NH_4^+-N + NO_3^--N) in soil was reduced by 14 %, whereas Olsen-P was increased by 8 % because of *Bt*-cotton. Root biomass yields were not different ($P > 0.05$), but root volume was significantly higher in *Bt* than non-*Bt* isoline. Time of sampling strongly ($P < 0.05$) affected the above parameters, showing their highest values at 60 or 90 DAS. A significant interactive effect of sampling time and treatments was also indicated. Our results suggest that *Bt*-cotton may constrain the availability of N, but enhances P-availability in these soils.

Introduction

In India, the area under transgenic crop cultivation has witnessed a phenomenal growth from 1.3 million hectares in 2005 to 3.8 million hectares in 2006, which is an increase of about 300 % in a year period (James 2006). Till March 2005, a total of 20 *Bt*-transgenic cotton hybrids have been released for commercial cultivation in northern, western and southern regions of India (Sharma et al. 2006). 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) (Dong and Li 2007). 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 (Motavalli et al. 2004). 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 (Motavalli et al. 2004).

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^{-1} soil) vs. 650 g ha^{-1} in case of *Bt*-corn crop, excluding grain (Blackwood and Buyer 2004). 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 (Dunfield and Germida 2004, Motavalli et al. 2004, O'Callaghan et al. 2005). As the rhizosphere (the zone directly surrounding and influenced by plant roots) contains a large

majority of biota populations of the soil, the plant–microbe interaction in the rhizosphere is one of the major factors regulating nutrient transformation and hence also the health and growth of plants. Therefore, any change to the quality of rhizosphere exudates can modify the biota composition in the soil (biodiversity) as well as their functions (Stotzky 2004, Patra et al. 2006, 2007). Although some research has examined the environmental impacts of the 'aboveground' portion of transgenic crops (O'Callaghan et al. 2005), relatively less research effort has examined the effects of these crops on microbially mediated processes and functions in soils (Bruinsma et al. 2003). There is also a paucity of information on the effects of transgenic cotton on nutrient availability in soil as an impact of altered rhizosphere activity. As adequate nutrient supply and efficient management are very important to harness and sustain the potential yields of *Bt*-cotton (Das et al. 2004, Blaise 2006), obviously there is a need to understand the nutrient dynamics in the rhizosphere soil of *Bt* and non-*Bt* cotton. Moreover, information about the impact of *Bt*-cotton on soil processes in India is very limited. In contrast to temperate regions, most Indian soils are poor in soil fertility (e.g. organic C < 1 %, N < 0.1 %). Thus, soil responses to *Bt*-crops may be different in India than that reported elsewhere. We hypothesize that *Bt*-cotton may adversely affect the availability of nutrients in soil. Therefore, the objective was to assess the dynamics of N and P availability in the rhizosphere of *Bt* and non-*Bt* cotton crops. We also examined the enzyme activities and their association with the availability of N and P in the rhizosphere soil.

Materials and Methods

Experimental site

This experiment was conducted in the *kharif* season (warmer rainy season, June–September 2005) under net house conditions at the Indian Agricultural Research Institute (IARI), New Delhi, located at 28°37′–28°39′N and 77°9′–77°11′E, which is about 250 m above mean sea level. The climate of Delhi is semi-arid and sub-tropical, characterized by hot summers and cold winters. The total precipitation during the study period was 431.1 mm and average temperatures ranged from 23.1 to 43.2 °C.

The soil (0–10 cm depth) used in this experiment was collected from the unfertilized and uncropped area (fallow for many years) of the IARI research farm. The soil is of alluvial origin, sandy clay loam in texture, alkaline in reaction and bears low cation exchange capacity (Table 1). It belongs to the hyperthermic family of Typic Haplustept.

Table 1 Initial characteristics of the experimental soil (air dry weight basis)

Parameters	Value
Sand (%)	47.4
Silt (%)	27.7
Clay (%)	24.8
Textural class	Sandy clay loam
pH (1 : 2.5 soil to water)	8.3
Electrical conductivity (1 : 2.5) (dS m^{-1})	0.24
CEC (cmol (p+) kg^{-1})	10.6
Total organic carbon (g kg^{-1})	6.8
Total N (g kg^{-1})	0.57
Ammonium N (mg kg^{-1})	10.29
Nitrate N (mg kg^{-1})	15.25
Total mineral N (mg kg^{-1})	25.54
Olsen-P (mg kg^{-1})	5.47

Treatments

The experiment was carried out with three treatments, viz. non-*Bt* cotton, *Bt* cotton and no-crop, and having three replications for each treatment. *Bt* and non-*Bt* cotton cultivars (MRC – 6301*Bt* and MRC – 6301) developed by Mahyco Research (India) were used for this study. *Bt*-cotton contained the *cry1Ac* gene and *nptII* and *aad* marker genes. The Government of India has approved the cultivation of these cultivars in the northern part of India in 2004. The crops were grown in porcelain pots (25 cm diameter and 35 cm height) using 15 kg soil. The pots were randomly arranged following the randomized block design. A basal dose of 0.58 g urea, 2.5 g single super phosphate and 0.66 g of muriate of potash (KCl) per pot (equivalent to 40 kg N, 26 kg P and 50 kg K ha^{-1} respectively) was applied (thoroughly mixed in 0–15 cm depth) at the time of sowing. Thereafter, first top dressing was carried out with 0.58 g urea (equivalent 40 kg N ha^{-1}) per pot after 30 days of sowing followed by second top dressing at 60 days after sowing (DAS) with same amount of N and 0.22 g muriate of potash (equivalent 16.5 kg K ha^{-1}) per pot. In each pot one plant was maintained until maturity. Irrigation and all other agronomic practices and guidelines were followed as recommended for growing this crop. Water stress was minimized with timely irrigation. There was no incidence of bollworm [*Helicoverpa armigera* (Hübner)] in *Bt* or non-*Bt* cotton during their growth.

Sampling and analysis of soil and plants

Destructive soil samples were collected at 0, 60, 90 and 120 DAS of cotton. At day 0 (just before seed sowing), the soil sample was taken immediately after fertilizer application (basal). Second sampling (60 DAS) was

carried out just before second top dressing. Soils adhering only to roots (0.15–0.20 kg) were collected to represent rhizospheric soils. Soils were removed from the pots by inverting them on a clean plastic sheet. The bulk soil of the pot was separated with hand as much as possible and discarded. Soils adhering only to the root surface were collected by gently jerking the roots on another clean plastic sheet. In this study, soil samples were collected from the rhizosphere only because the effect of *Bt*-toxin released from the roots is expected to be highest in this zone. We assumed that N movement and contribution from bulk soil is similar for both *Bt* and non-*Bt* crops under the present experimental conditions. Fresh soil samples were sieved using a 2 mm sized mesh (aggregates larger than 2 mm were broken and soil was removed as efficiently as possible from the root surface so that only stones and large roots were discarded), homogenized and stored in the refrigerator at 4 °C.

Analysis of the soil samples was completed in 3–4 days. The gravimetric moisture content was determined before storing the samples at 4 °C. The total root mass from each pot was also collected carefully at every sampling event and washed successively with tap water followed by distilled water (Patra et al. 2004). At maturity, the number of bolls from each plant and their weights were recorded.

Dehydrogenase activity was determined by monitoring the rate of triphenyl formazon (TPF) production from triphenyl tetrazolium chloride (TTC), using the method of Klein et al. (1971). Soil respiration was estimated by measuring oxygen consumption by soil in a Warburg flask incubated at 28 ± 1 °C, as described by Umbreit et al. (1972). Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were estimated by the methods described by Keeney and Nelson (1982). Soil was extracted with 2 M KCl and distilled in a Kjeldahl semi automatic distillation apparatus (Gerhardt Vapodest 30, Königswinter, Germany) for determining NO_3^--N and NH_4^+-N. 0.5 M $NaHCO_3$ (pH 8.5) extractable P was determined colorimetrically by the ammonium molybdate blue colour method (Olsen and Sommers 1982). Root volume was determined by the water displacement method (Pushpadas 1979). The root biomass weight was determined after drying the roots for 24 h at 60–70 °C in an oven. At maturity, the total number of bolls and the boll size (weight) were recorded.

Statistical analysis

Analysis of variance (ANOVA) (two-way or one-way) was performed to determine the effects of treatments (*Bt*, non-*Bt* and no-crop) and time (days) on soil enzymes and the availability of nutrients, as well as the root characteristics of cotton, using the procedure suggested by Gomez and Gomez (1984) for randomized block design, using MICROSOFT EXCEL (Microsoft Corporation, Redmond, WA, USA) and/or SPSS (window version 12.0; SPSS Inc., Chicago, IL, USA). Least significance difference (LSD) or Duncan's multiple range test (DMRT) at P = 0.05 was used to determine whether the means differed significantly.

Results and Discussion

Dehydrogenase activity and soil respiration

Dehydrogenase activity (Table 2) was highest in the non-*Bt* treatment (31.3 μg TPF g^{-1} h^{-1}), followed by *Bt*-cotton (26 μg TPF g^{-1} h^{-1}), and least in the no-crop treatment (23.7 μg TPF g^{-1} h^{-1}). It also varied significantly between the four sampling times (20.2–27.3 μg TPF g^{-1} h^{-1}). Dehydrogenase activity reflects the oxidative activity or intensity of metabolism of soil microflora and can be used as an indicator of microbial activity or populations in soils (Nannipieri et al. 2003). The lower dehydrogenase activity in rhizosphere soils under *Bt*-cotton is in conformity with the results of Wei et al. (2006) and Donegan and Seidler (1999). Wu et al. (2004) also reported reduced dehydrogenase activity during the decomposition of straw from *Bt*-transgenic rice cultivars under flooded conditions in China. However, Shen et al. (2006) observed no differences in the dehydrogenase activity in the rhizosphere of *Bt*-cotton.

Lower dehydrogenase activity in the *Bt*-system indicates that a portion of the organisms was perhaps inhibited and did not participate in the metabolic activities of the soil (Masto et al. 2006). It may have been partly because of unfavourable conditions in the rhizosphere under *Bt*-cotton or because of a negative effect of *Bt*-toxins on certain microbial groups, which might have retarded metabolic activities in the soil.

Average soil respiration rate (Table 3) was also significantly higher in non-*Bt* cotton (7.06 μl O_2 g^{-1} h^{-1}) followed by *Bt*-cotton (6.81 μl O_2 g^{-1} h^{-1}) and no-crop (6.59 μl O_2 g^{-1} h^{-1}) treatments. The highest value of soil

Table 2 Dehydrogenase activity (μg TPF g^{-1} h^{-1}) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	20.7	33.0	43.4	28.0	31.3
BT	19.4	33.0	22.8	28.6	26.0
NC	20.5	27.8	21.1	25.3	23.7
Mean	20.2	31.3	29.1	27.3	

LSD (P = 0.05): treatment (T) = 1.90; days (D) = 2.20; T × D = 3.81.

Table 3 Soil respiration ($\mu l\ O_2\ g^{-1}\ h^{-1}$) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	6.10	7.46	7.78	6.91	7.06
BT	6.20	7.22	6.75	7.07	6.81
NC	6.30	6.88	6.39	6.78	6.59
Mean	6.20	7.19	6.97	6.92	

LSD (P = 0.05): treatment (T) = 0.09; days (D) = 0.11; T × D = 0.19.

respiration rate was observed at 90 DAS with non-*Bt* cotton, and was significantly reduced thereafter. Soil respiration is a useful index of the overall biological activity in soil (Nannipieri et al. 2003) and is a critical determinant of ecosystem C storage. In this study, soil respiration rates followed the trend of dehydrogenase activity. Stotzky (2004) reported that CO_2 evolved from soil amended with ground *Bt* (Cry1Ab) maize biomass was significantly lower than that of the soil amended with unmodified isogenic maize biomass. The mechanisms by which the presence of the Cry1Ab toxin might depress microbial activity are unclear (Rui et al. 2005, Griffiths et al. 2006). It is likely that the insertion of the Cry1Ac gene into cotton might alter either the amount or character of one or more root exudates important to micro-organisms in the rhizosphere, thereby reducing the respiratory activity. It might also alter the composition and conformation of some components in cotton, such as lignin, cellulose and hemicellulose, which protect the associated polysaccharides and proteins from microbial decomposition (Saxena and Stotzky 2001). The present results suggest that the significant differences in soil dehydrogenase activity and soil respiration may be due to the quality of root composition and root exudates. However, further investigations into the structure and composition of *Bt*-cotton root exudates and changes in soil microbial community composition are necessary to confirm this hypothesis.

N and P availability

Although NH_4^+-N content in the soil samples was variable in the range of 10–25 mg kg^{-1} (Table 4), the effect of the treatments was not significant. There was an increase in soil NH_4^+-N from 0 to 60 DAS, but then decreased significantly at 90 DAS. The concentration of NH_4^+-N at 90 and 120 DAS was statistically similar.

In case of NO_3^--N in soil (Table 5), a significant reduction (13.60 mg kg^{-1}) was noticed in soils under *Bt* cotton in comparison with that of the non-*Bt* (17.57 mg kg^{-1}) and no-crop (17.19 mg kg^{-1}) treatments. Though an increase in NO_3^--N in the soil was evident at 60 DAS

Table 4 Ammonium N (mg kg^{-1}) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	10.11	23.61	23.51	20.00	19.31
BT	10.28	23.73	17.78	20.30	18.02
NC	10.47	25.36	20.08	22.78	19.67
Mean	10.29	24.23	20.46	21.03	

LSD (P = 0.05): treatment (T) = not significant; days (D) = 3.16; T × D = 5.48.

Table 5 Nitrate N (mg kg^{-1}) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	15.16	38.10	9.78	7.25	17.57
BT	15.27	21.87	9.90	7.35	13.60
NC	15.31	18.09	18.28	17.08	17.19
Mean	15.25	26.02	12.65	10.56	

LSD (P = 0.05): treatment (T) = 2.95; days (D) = 3.40; T × D = 5.90.

when compared with that of the initial 0 DAS, at the next sampling event it decreased significantly. NH_4^+-N and NO_3^--N content of soil did not differ significantly at 120 DAS when compared with that of 90 DAS. Except at 60 DAS, all other sampling events were similar for their NO_3^--N contents.

The total mineral nitrogen (NH_4^+-N + NO_3^--N) in soil among different treatments in this study maintained a trend similar to that of NO_3^--N. As shown in Table 6, non-*Bt* cotton (36.88 mg kg^{-1}) and no-crop (36.75 mg kg^{-1}) treatments showed no significant difference in the mineral nitrogen content, but *Bt*-cotton (31.56 mg kg^{-1}) was found to maintain a significantly lower mineral nitrogen than the other two treatments. Reduction in the mineral N content under *Bt*-cotton was as much as 14 % when compared with that of the non-Bt isoline (Fig. 1). There has been paucity of information regarding the effects of transgenic crops on the availability

Table 6 Total mineral N (mg kg^{-1}) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	25.27	61.70	33.30	27.25	36.88
BT	25.30	45.60	27.69	27.65	31.56
NC	25.34	43.44	38.36	39.86	36.75
Mean	25.30	50.25	33.12	31.58	

LSD (P = 0.05): treatment (T) = 3.55; days (D) = 4.10; T × D = 7.11.

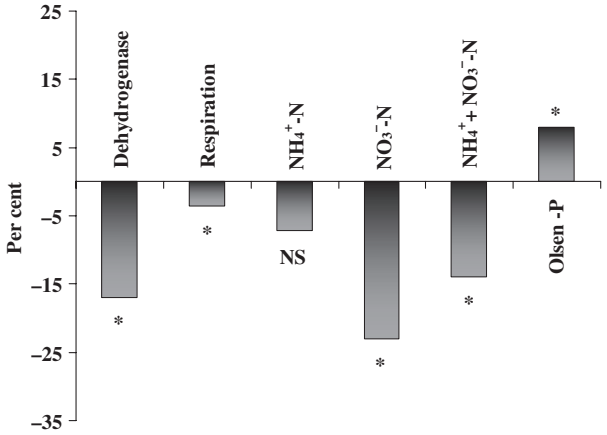

Fig. 1 Impact of *Bt*-cotton on soil enzyme activities and availability of N and P in the rhizosphere soil. Information is based on the comparison between *Bt* and non-*Bt* cotton cultivars. NH_4^+-N: ammonium N; NO_3^--N: nitrate N; TMN: total mineral N. For percent, calculation was as follows: [(Value in *Bt* cotton–value in non-*Bt* cotton)/value in non-*Bt* cotton] × 100. *Significant at P = 0.05 (n = 12). Significance was derived from the difference of means (*Bt* vs. non-*Bt*) of each parameter and in comparison with the least significant difference (LSD) (P = 0.05) value (given in Table 1 for each parameter).

of soil nutrients. 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. 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 the mechanisms for these effects is still unclear (Icoz and Stotzky 2008). Nitrogen immobilization by soil organisms, during the decompositions of fine dead root biomass, under *Bt*-cotton may be a reason for the reduced available N content in the soil. Reduced

Table 8 Olsen-P (mg kg^{-1}) in the rhizosphere soils under *Bt* (BT) and non-*Bt* (NBT) cotton crops. NC indicates no crop

	Crop growth period (days)				
Treatments	0	60	90	120	Mean
NBT	5.40	13.50	5.95	5.81	7.66
BT	5.80	9.93	9.01	8.43	8.29
NC	5.20	11.44	9.72	11.99	9.59
Mean	5.47	11.62	8.23	8.74	

LSD (P = 0.05): treatment (T) = 0.41; days (D) = 0.48; T × D = 0.83.

dehydrogenase activity and soil respiration rate and its strong association (Table 7) with mineral N also indicate the possibility of reduced N mineralization in the rhizosphere of *Bt*-cotton.

Unlike mineral N, available P (i.e. Olsen-P) content of soil was maintained at a significantly higher level in the case of *Bt*-cotton (8.29 mg kg^{-1}) than non-*Bt* cotton (7.66 mg kg^{-1}) (Table 8), whereas soils under no-crop were found to maintain in highest available P content. The average increase in the availability of P under *Bt*-cotton was 8 % when compared with that of the non-*Bt* counterpart (Fig. 1). As in the case of mineral N, available P was highest at 60 DAS and thereafter decreased significantly. Interestingly, at 120 DAS Olsen-P content was again increased.

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 example of factors affecting soil P 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.

Table 7 Pearson's correlation (r) matrix for root parameters and soil nutrient availability during the growth period of cotton (*Bt* and non-*Bt*) crop

Properties	Root volume	Root dry matter	Dehydrogenase activity	Soil respiration	NH_4^+-N	NO_3^--N	TMN	Olsen-P
Root volume	1	0.98**	ns	ns	−0.54*	−0.85**	−0.89**	−0.60**
Root dry matter		1	ns	ns	−0.55*	−0.87**	−0.91**	−0.69**
Dehydrogenase activity			1	0.92**	0.57**	ns	0.38*	ns
Soil respiration				1	0.72**	ns	0.50**	0.37*
NH_4^+–N					1	ns	0.57*	ns
NO_3^-–N						1	0.97**	0.86**
TMN							1	0.80**
Olsen-P								1

NH_4^+-N: ammonium N; NO_3^--N: nitrate N; TMN: total mineral N; Olsen-P: 0.5 m $NaHCO_3$ extractable P
*, ** Correlations significant at P < 0.05 and 0.01 respectively (n = 18). ns, not significant at P < 0.05.

2000). Citrate and oxalate appear to be the most efficient components of root exudates with respect to mobilization of P from soils low in readily available P (Jones 1998). 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. Thus it can be presumed that P availability was enhanced because of changes in rhizospheric conditions under *Bt*-cotton. This result 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 our study (Table 7), indicating that enhanced availability of P might not only be due to variation in root exudates, but perhaps also due to rhizospheric micro-organisms. Further investigation is needed to characterize root exudates produced by transgenic cotton cultivars in order to clearly identify the 'cause and effect' mechanism, for its ability to improve soil P release, which may prove valuable for efficient P management in the agricultural soils in this region.

Root and cotton yield parameters

Root biomass was not significantly different between the two cultivars (*Bt* and non-*Bt*) during their growth. Root volume was similar at the initial growth stage, i.e. at 60 DAS (Fig. 2a), but at 60 and 90 days there were significant differences between *Bt* and non-*Bt* cultivars.

Root characteristics are important factors that influence the rhizosphere biochemistry and the transformation of nutrients. It is important in the case of *Bt*-crops, because one of the major avenues of *Bt*-toxin release to the soil is through root exudates. The range of root parameters observed in this study supports earlier observations (Foshee et al. 1999, Rosolem et al. 1999). In this study root parameters had significantly negative relationships with the available N and P contents in the rhizosphere soil (Table 7), indicating higher nutrient depletion with increasing root biomass or volume. There is also a possibility of reduced N and P mineralization (or high immobilization) because of changes in the chemical composition of root or microbial properties in the rhizosphere of *Bt*-cotton.

During crop harvest, the number of bolls per plant varied from 19 to 23 and the weight of each boll was in the range of 3.9–4.5 g (data not presented). However, such differences between the cultivars were not significant. One reason for similar performances may be due to no incidence of bollworm in the non-*Bt* cultivar during crop growth. Lower levels of availability of mineral N in soils under *Bt*-cotton may be another reason, which restricted better performance by the *Bt* cultivar than the non-*Bt* near-isoline.

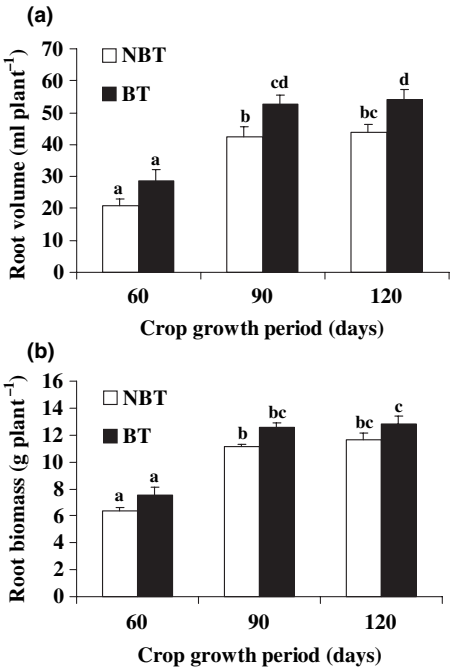

Fig. 2 (a) Root volume and (b) root biomass of *Bt* and non-*Bt* cotton crops at different growth stages. Bars indicate standard errors (n = 3). Means followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's multiple range *post-hoc* test (DMRT).

Conclusions

Based on the results it can be concluded that *Bt*-cotton may have negative effects on dehydrogenase activity, soil respiration rate and availability of total mineral N (NH_4^+-N + NO_3^--N) in rhizosphere soils. However, it may be mentioned that for mineral–N, the results are applicable for urea fertilizer only. In contrast, there is a possibility of increasing Olsen-P. Time of sampling during plant growth also strongly ($P < 0.05$) affected the above parameters, most of which were at their highest values at 60 and 90 DAS. There was also a significant interactive effect between sampling time and treatments. However, more studies are needed to find out more about enzyme activities and the availability of reduced and increased levels of N and P in the soils under *Bt*-cotton and develop strategies for the efficient management of these nutrients in the fields.

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