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Evaluating safety of genetically modified crops: effect of *Bt*-transgenic cabbage plants on microbial dynamics and dehydrogenase activity*

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Insecticidal proteins of *Bacillus thuringiensis* (*Bt*) have emerged as the proteins of choice to be expressed in transgenic (genetically modified, GM) crops towards an environment-friendly mode of insect pest management in agriculture (Kumar 2003, Paul *et al*. 2005). Among the commercial GM crops, transgenic crops carrying *Bt* gene have outnumbered the crops carrying other genes. Since root exudates of transgenic plant have insecticidal *Bt*K protein (Prescott *et al*. 1990, Saxena *et al.* 2002), there have been concerns about the effect of such proteins on soil flora and fauna (Glare and Callaghan 2002, Wu *et al.* 2004).

The environmental impacts of genetically modified plants are still largely unknown and are often unresolved (Hails 2000, Gray 2004 Snow *et al*. 2005). *Bt*-transgenic plants might have direct or indirect impacts on different aspects of ecosystems. Soil microbial diversity may be one of the important aspects to study the impact of GM plants. The essential role played by soil microorganisms in nutrient cycling and in organic matter decomposition is well known. It is therefore crucial to investigate the possible impacts of *Bt*-transgenic plants on soil micro-flora before their environmental release.

In the present investigation, the rhizospheric soil samples from transgenic tropical cabbage (*Brassica oleracea* var. capitata), carrying a *Bt*-gene for control of diamond back moth (DBM, *Plutella xylostella*), was collected at three different times with an interval of 25 days and the number of culturable bacteria, phosphate-solubilizing bacteria (PSB), actinomycetes and fungi was counted by plate count method and compared to those from non-transgenic one. Soil dehydrogenase activity was also measured to analyze the variation between transgenic and non-transgenic cabbage. It was hypothesized that the numbers and activity of culturable microbial communities would be altered in the presence of root exudates of transgenic cabbage.

The seeds of non-transgenic and transgenic lines of tropical cabbage were procured from the National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute (IARI), New Delhi, India during 2001–2. Seedlings of both the lines (non-transgenic and transgenic) were grown in half strength Murashige and Skoog (MS) medium with vitamins B_5 and transplanted in pots (one seedling/pot) containing surface soil (0–15 cm) of a field, untreated with pesticides or fertilizer, at IARI, New Delhi. In a separate experiment, properties of the soil used in the experiment were determined using the standard methodologies. At 25, 50 and 75 days after transplanting (DAT), the plants of both the lines were uprooted separately, the excess soil was removed by shaking and the roots with adhered rhizosphere soil were placed into water (*p*H 7) for extracting the representative microbial population from the soil and the root surface. The flasks were shaken well and 10-fold serial dilutions were made. 1 ml of sample from suitable dilutions was plated separately on Nutrient agar, Pikovaskaya, Ken Knight's and Martin's Rose Bengal medium for growing bacteria, phosphate solubilizing bacteria, actinomycetes and fungi respectively, expected to present in the rhizospheric soil. The plates were incubated at 30°C for three days in the case of bacteria and fungi and for seven days in the case of actinomycetes. Dehydrogenase activity was determined by using 3% solution of 2, 3, 4-triphenyl tetrazolium chloride and was measured in a UV-Vis spectrophotometer at a wave length of 485 nm. Microbial counting and enzyme activity analysis were carried out with the rhizospheric soil of experimental plot, conducted in completely randomized design (CRD). Univariate analysis of variance for testing interaction among treatment means (non-transgenic and

* Short note

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Table 1 Enumeration of culturable microorganisms present in the rhizosphere of *Bt*. Transgenic (T) and non-transgenic (N) cabbage

Sampling Date	Plant type	Bacteria	Actinomycetes	Fungi	PSB
Count		Average(±S D) cfu × 10^8	Average(±S D) cfu × 10^5	Average(±S D) cfu × 10^4	Average(±S D) cfu × 10^4
25	T	14.333 (3.055)	6.667 (1.527)	4.667 (1.527)	16.000 (4.000)
	N	18.000 (7.937)	4.333 (2.081)	3.333 (1.527)	12.667 (2.081)
50	T	23.000 (6.000)	11.000 (4.582)	5.333 (3.055)	4.667 (1.527)
	N	37.667 (5.507)	15.667 (3.511)	4.667 (2.516)	10.000 (7.000)
75	T	20.667 (8.504)	19.333 (4.041)	10.000 (3.464)	42.333 (2.516)
	N	23.000 (8.185)	14.667 (3.214)	19.333 (2.516)	52.667 (3.785)

* T = Transgenic; N, non-transgenic

*Standard deviation (±S D) is given in parentheses is after average value

transgenic) was carried out using SPSS program (version 10.0) to interpret the effect of transgenic cabbage on the number of representative rhizospheric microorganisms and dehydrogenase activity.

The experimental soil was found to be sandy-loam type having *p*H 8.4, with organic carbon 0.39%, cation exchange capacity (CEC) - 8.57 C mol(p^+)/kg, electrical conductivity (EC) – 0.32 ds/m, available nitrogen – 225 kg/ha, available phosphorus - 16.98 kg/ha, available potassium - 237 kg/ha and organic phosphorus – 155.7 mg/kg of soil.

The enumeration of microorganisms showed that the bacterial population was highest (10^8 cfu) as compared to the count of other microorganisms in the rhizosphere soil from both transgenic and non-transgenic cabbage (Table 3). The bacterial population was higher in the rhizosphere of non-transgenic cabbage on all the three days of observation. The population of bacteria in rhizophere soils of both non-transgenic and transgenic plants increased up to 50 DAT and then declined up to 75 DAT to a level which was more than the initial count recorded on 25 DAT (Table 1). Statistically significant difference on bacterial population was observed at 5% level with respect to dates (25, 50 and 75) and lines (non-transgenic and transgenic), however, non-significant results was observed on interaction between dates and lines and variation of data between the replicates (Table 3).

In the case of actinomycetes population, at 25 DAT, no significant difference on numbers was observed between the rhizosphere of two lines, but it got progressively increased with the time in transgenic cabbage roots (Table 1). The statistical analysis (Table 3) showed that the three dates of sampling had significant role in the growth of actinomycetes population.

Table 2 Dehydrogenase activities in the rhizosphere of transgenic and normal cabbage

Sampling date	25 DAT	50 DAT	75 DAT
Count	Average (±S D) µl H_2 24/h/g	Average (±S D) µl H_2 24/h/g	Average (±S D) µl H_2 24/h/g
Transgenic cabbage	0.026 (0.0015)	0.027 (0.0011)	0.028 (0.0005)
Non-transgenic cabbage	0.022 (0.0062)	0.029 (0.0051)	0.034 (0.0066)

The number of fungi, enumerated initially at 25 DAT, was found to be higher in the rhizospheric soil of transgenic cabbage than those of non-transgenic one. The fungi community increased with time in both transgenic and non-transgenic cabbage and was found to be highest on 75 DAT. Statistically significant difference on fungal population was observed at 5% level with respect to dates (25, 50 and 75) but with respect to lines it was found to be non-significant.

The initial population of PSB at 25 DAT in the rhizospheric soil of transgenic line was found to be more than that of non-transgenic cabbage. The number of PSB decreased at 50 DAT and then suddenly increased towards 75 DAT. Final PSB population at 75 DAT was higher in the rhizosphere of non-transgenic cabbage than in transgenic (Table 1). Statistical analysis showed no significant difference is there due to lines (Table 3). However, the effect of date and interactions between date and lines on PSB population was significant at 5% level of significance.

The total dehydrogenase activity (Menon, *et. al.* 2005) is a function of the number of living organisms present in the soil. While there was no significant difference in the dehydrogenase activity on 25 and 50 DAT around the rhizosphere of both types of cabbage, it appeared to be higher in the rhizosphere of non-transgenic cabbage on 75 DAT. This can be explained as the communities of bacteria, fungi and phosphate-solubilizing bacteria were relatively higher around the roots of non-transgenic cabbage on 75 day after transplantation due to the effect of root exudate. The effect of sampling date was found to be significant but not of lines.

Except the number of bacteria, all other microorganism in rhizospheric soil was found to be lower around transgenic plant than those of non-transgenic one, however, such differences were not statistically significant. Such observation could also be linked with the lower dehydrogenase activity in rhizosphere of transgenic plant. The populations of

Table 3 Univariate analysis of variance for five different dependent variable

Source	Df	*Mean square	F statistic	Probability
Dependent variable: Bacteria				
DAT	2	305.056	12.859	0.002
Variety	1	213.556	9.002	0.013
DAT × variety	2	68.722	2.897	0.102
R Squared = 0.978 (adjusted R squared = 0.961)				
Dependent variable: Actinomycetes				
DAT	2	207.056	20.322	0.000
Variety	1	2.722	0.267	0.616
DAT × variety	2	35.389	3.473	0.072
R Squared= 0.968 (adjusted R squared = 0.942)				
Dependent variable: Fungi				
DAT	2	208.222	28.054	0.000
Variety	1	26.889	3.623	0.086
DAT × variety	2	53.556	7.216	0.011
R squared = 0.958 (adjusted R squared = 0.924)				
Dependent variable: PSB				
DAT	2	2762.389	170.987	.000
Variety	1	76.056	4.708	0.55
DAT × variety	2	71.722	4.439	.042
R squared = 0.990 (adjusted R squared = 0.981)				
Dependent variable: Dehydrogenase				
DAT	2	7.017e-05	82.367	0.000
Variety	1	5.556E-06	0.261	0.696
DAT × variety	2	3.672E-05	1.727	0.227
R squared = 0.990 (adjusted R squared = 0.981)				

*Mean square = sum of square / degrees of freedom (Df)

microorganism are dynamic and may affect each other.

SUMMARY

The fear that *Bt* transgenic cabbage may secrete some toxic or inhibitory substance that will adversely affect the populations of microorganisms dwelling in its rhizosphere did not appear to be true. Though only the culturable microbial population constituting less than 1% of the soil bacteria was examined, the study proved that *Bt* transgenic was not adversely affecting the representative culturable microorganisms dwelling around their rhizosphere in soil.

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