

## Estimation of Bt protein (Cry1Ac) concentration in Bt and Non Bt soils

E. Ramachandran (Scientist, JK Agri Genetics Ltd.), using Enviroligix Quantiplate TM Kit for Cry1Ab/Cry1Ac at Institute of Microbial Technology (IMTECH) Chandigarh has done estimation of Bt protein (Cry1Ac) concentration in Bt and Non-Bt soils for I crop season. The estimation of Bt protein (Cry1 Ac) concentration in soil samples of II crop season was done using Cry1 Ac/ Ab Elisa Kit (Amar Immunodiagnostics), provided by JK Agri Genetics Ltd. The level of Cry1Ac protein in all soil samples was evaluated using Enzyme linked immunosorbent assays (ELISA).

### Procedure:

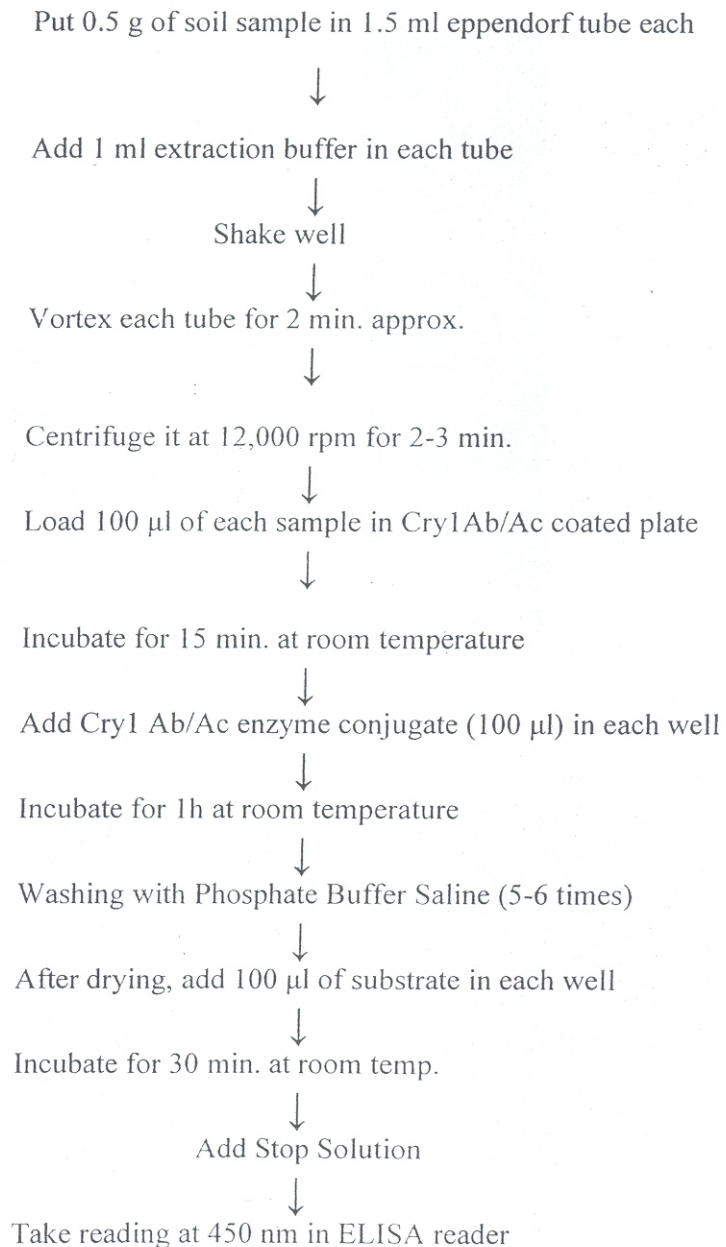


Table 1 represents the readings of blank, 1.5ng, 10ng and 25ng (calibrators available in kit for CryI Ac proteins in triplicate) and soil samples i.e. control (0 day), Bt, Bt rhizosphere, Non-Bt and Non-Bt rhizosphere (50 and 150 days) of Rajasthan, Gujarat, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu, of I crop season.

Table 2 represents the readings of blank, negative and positive control (available in kit for CryI Ac proteins in duplicate) and soil samples i.e., control (0 day), Bt rhizosphere and Non-Bt rhizosphere (50 and 150 days) of Rajasthan, Gujarat, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu, of II crop season.

It was observed that there was no detectable amount of CryI Ac protein present in the soil samples of both the crop seasons.

## **Conclusions**

On the basis of present study, it can be concluded that the soil microbial diversity data on total colony counts of culturable microorganisms revealed no particular difference in control (0 day), Bt, Bt rhizosphere, Non-Bt and Non-Bt rhizosphere (50 and 150 days) of I crop season and control (0 day), Bt rhizosphere and Non-Bt rhizosphere (50 and 150 days) of II crop season of any of the state i.e. Rajasthan, Gujarat, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. About 700-800 bacterial cultures from control, Bt, Bt rhizosphere, Non-Bt and Non-Bt rhizosphere soil samples of both the crop seasons were isolated on tryptone soya agar (TSA) and preserved as glycerol stocks at  $-70^{\circ}\text{C}$ . The unique colony characteristics for all the isolated colonies have been recorded and the most predominant cultures from TSA plates have been identified. The predominant types of colonies obtained on TSA plates of control, Bt, Bt rhizosphere, Non-Bt and Non-Bt rhizosphere soil samples of I and II crop season belonged to the genera of *Bacillus* sp. The Bt protein (CryI Ac) concentration in Bt and Non-Bt soil samples estimated and it has been observed that there was no detectable amount of CryI Ac protein in the soil samples of both the crop seasons.

**Table 1: Results\* of estimation of Bt protein (Cry1 Ac) concentration in Control (0 day), Bt, Bt rhizosphere, Non-Bt and Non-Bt rhizosphere (50 and 150 days) soil samples of I crop season, using Envirologix Quantiplate™ Kit for Cry1 Ab/Cry1 Ac.**

Blank	1.5 ng*	10 ng*	2.5 ng*
0.000	0.192	0.880	1.727
	0.181	0.898	1.712
	0.171	0.916	1.703

States Soil Samples	Rajasthan			Gujarat			Maharashtra		
	0 d*	50 d	150 d	0 d	50 d	150 d	0 d	50 d	150 d
Control	-0.005			0.006			-0.038		
Bt		0.019	0.000		0.008	-0.015			
Bt rhizosphere		-0.027	-0.002		-0.008	-0.008		-0.024	-0.010
Non-Bt		-0.008	-0.002		-0.006	-0.003		-0.023	-0.010
Non-Bt rhizosphere		-0.007	0.020		-0.005	0.016		-0.014	-0.020
								-0.013	-0.010

States Soil Samples	Karnataka			Andhra Pradesh			Tamil Nadu		
	0 d	50 d	150 d	0 d	50 d	150 d	0 d	50 d	150 d
Control	-0.010			-0.006			0.005		
Bt		-0.003	-0.017		-0.000	-0.007			
Bt rhizosphere		0.026	-0.009		-0.002	-0.007		-0.020	-0.030
Non-Bt		-0.018	-0.007		-0.004	-0.015		-0.007	-0.013
Non-Bt rhizosphere		-0.017	0.005		0.002	0.014		0.000	-0.007
								0.000	-0.002

\* , Values are reading at 450 nm wavelengths in ELISA reader

\* , Calibrators available in kit for Cry1 Ac in triplicates  
d: Number of days

**Table 2: Results\* of estimation of Bt protein (Cry1 Ac) concentration in Control (0 day), Bt rhizosphere and Non-Bt rhizosphere (50 and 150 days) soil samples of II crop season, using Cry1 Ac/ Ab Elisa Kit.**

Blank	Negative Control*	Positive Control*
0.000	-0.007	F.FFF
	0.003	F.FFF

States Soil Samples	Rajasthan			Gujarat			Maharashtra		
	0 d	50 d	150 d	0 d	50 d	150 d	0 d	50 d	150 d
Control	-0.010			-0.006			0.016		
Bt rhizosphere		-0.004	0.001		0.002	0.017		-0.008	0.000
Non-Bt rhizosphere		-0.002	-0.010		-0.013	-0.008		-0.001	0.000

States Soil Samples	Karnataka			Andhra Pradesh			Tamil Nadu		
	0 d	50 d	150 d	0 d	50 d	150 d	0 d	50 d	150 d
Control	0.000			-0.010			0.007		
Bt rhizosphere		-0.005	-0.001		0.016	-0.008		-0.000	-0.009
Non-Bt rhizosphere		0.002	0.003		-0.010	0.019		-0.004	0.000

\* , Values are reading at 450 nm wavelengths in ELISA reader  
 \* , Negative Control & Positive Control available in kit for Cry1 Ac  
 d: Number of days  
 FFF: Beyond range

**Compositional Analysis:**

The compositional analysis of JKAL Bt Cotton hybrids, specific to Central zone, studied at Vitro Labs Hyderabad

S.No	Hybrids	Total Protein	Oil	Reducing Sugar
1	JK-Varun Non-Bt	24.66	18.59	2.90
2	JK-Varun Bt	25.02	18.36	3.00
3	JKCH-99 Non-Bt	22.67	18.07	3.10
4	JKCH-99Bt	23.19	19.04	3.42

**Compositional Analysis :**

The compositional analysis of all JKAL Bt Cotton hybrids studied at Vitro Labs Hyderabad

S.No	Hybrids	Total Protein	Oil	Reducing Sugar
1	JKCH1050 Non- Bt	25.39	16.14	2.65
2	JKCH 1050 Bt	24.89	14.25	3.00
3	JKCH1947 Non-Bt	22.44	19.38	3.20
4	JKCH 1947 Bt	23.64	13.46	3.50

**Compositional Analysis:**

The compositional analysis of JKAL Bt Cotton hybrids, specific to South zone, studied at Vitro Labs Hyderabad

S.No	Hybrids	Total Protein	Oil	Reducing Sugar
1	JK-Durga Non-Bt	24.37	18.51	3.60
2	JK-Durga Bt	24.52	16.90	3.30
3	JKCH-99 Non-Bt	22.67	18.07	3.10
4	JKCH-99Bt	23.19	19.04	3.42