Resistance to Bt in Indian *Helicoverpa armigera*

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Bt cotton targets

- Cotton bollworm ***Helicoverpa armigera***

  181 host plants - 69 crop species

  losses - US $ 540 mill annually

- Spiny bollworms - *Earias insulana* and *E. vitella*
- Pink bollworm - *Pectinophora gossypiella*

Bt cotton in India

- Total cotton area 8 mill ha (70% G.hirsutum)
- Farmers planted illegal Bt cotton over large areas up to 2001
- 2002 - Bt Cry1Ac hybrids commercialised in a joint venture Mhyco-Monsanto. Now several other companies.
- Registered hybrids suitable for the centre and south, not irrigated north
- Hybrid cotton – 40% of the cotton area

BT cotton area

- 2003 – c.1.2% of total cotton
- 2004 – c.6% of total cotton (11% of hybrids)

- Still as much unregulated Bt (inc F2 and F3) as 'official' cotton

Tissue expression in plants

In-season changes in Cry1Ac expression (µg/g fresh weight) in the upper canopy leaves of Bt-cotton hybrids –2003

	Bt Bollgard® hybrids																
DAS	**RCH-2**		**RCH-20**		**RCH-134**		**RCH-138**		**RCH-144**		**MECH-12**		**MECH-162**		**MECH-184**		**LSD**
30	5.15	± 0.9abc	6.61	± 1.1d	4.67	± 0.7a	4.42	± 0.3a	5.56	± 0.5abcd	6.20	± 1.0ab	4.97	± 0.7bcd	6.47	± 1.6cd	1.37
58	3.43	± 0.3bc	3.60	± 0.3bc	2.33	± 0.4a	3.91	± 0.4c	2.96	± 0.5ab	4.05	± 0.4c	2.68	± 0.5a	3.57	± 0.6bc	0.64
70	2.23	± 0.9bcd	1.94	± 0.8abc	1.26	± 0.5a	2.91	± 0.5de	1.87	± 0.2abc	3.32	± 0.8e	1.35	± 0.6ab	2.51	± 0.5cde	0.94
85	1.46	± 0.8	2.40	± 1.1	1.53	± 0.9	2.62	± 1.2	1.63	± 0.3	2.31	± 1.2	1.16	± 0.3	2.60	± 0.8	NS
95	0.58	± 0.9	0.77	± 0.5	1.66	± 0.1	0.97	± 1.2	1.11	± 1.0	1.07	± 0.4	0.55	± 0.4	0.89	± 0.7	NS
110	0.21	± 0.1ab	0.41	± 0.2bc	0.22	± 0.1ab	0.14	± 0.1a	0.44	± 0.2c	0.38	± 0.2bc	0.16	± 0.1a	0.47	± 0.2c	0.21
124	0.09	± 0.1	0.03	± 0.0	0.07	± 0.1	0.15	± 0.1	0.13	± 0.2	0.32	± 0.3	0.02	± 0.0	0.26	± 0.2	NS
138	0.02	± 0.0a	0.01	± 0.0a	0.01	± 0.0a	0.42	± 0.2b	0.49	± 0.3b	0.21	± 0.1a	0.01	± 0.0a	0.71	± 0.1c	0.20
148	0.01	± 0.0	0.01	± 0.0	0.10	± 0.2	0.03	± 0.0	0.02	± 0.0	0.08	± 0.0	0.01	± 0.0	0.12	± 0.1	NS

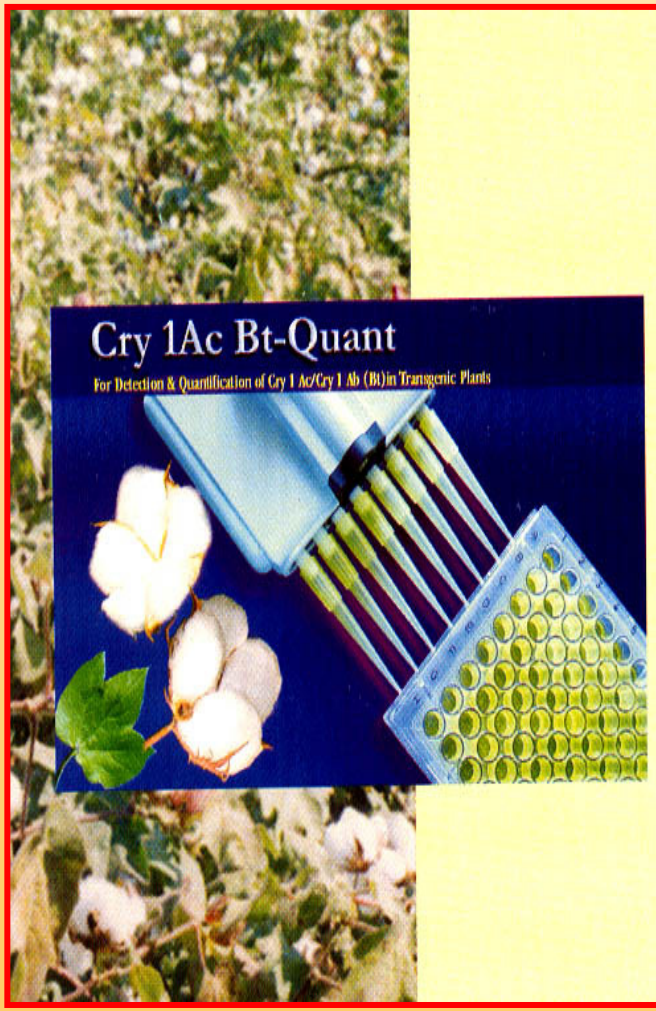
Abbreviations: DAS = Days after sowing; LSD = Least Significant Difference.
Numbers followed by different letters within a row are significantly different at P= >0.05.

- Expression quite variable amongst hybrids
- Toxin expression in leaves>buds>bolls>flowers
- Late season and old tissue – low expression
- *H.armigera* survival increases below 1.8µg/g tissue
- Toxin in boll rind, buds and flowers inadequate in late season (>110 days)

Quantitative ELISA

Cry1Ac:	**Detection sensitivity: 10 ppb**
Cry2Ab:	**Detection sensitivity: 20 ppb**
Pyrethroids:	**Detection sensitivity: 2 ppb**
Endosulfan:	**Detection sensitivity: 5 ppb**

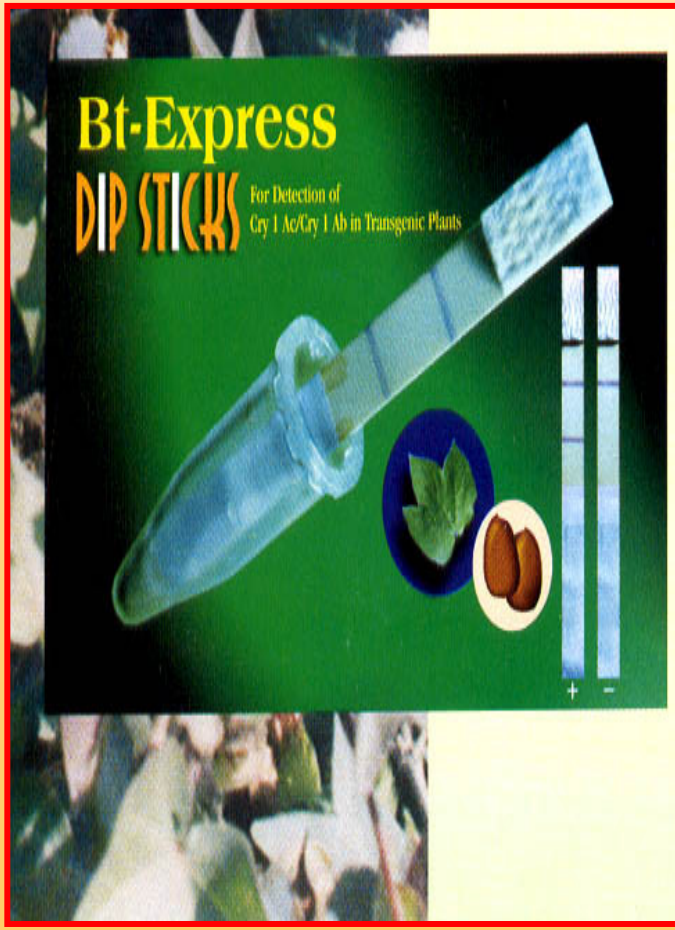
Bt-Quant



Cry 1Ac Bt-Quant

1. The Cry1AC Bt -Quant is an ELISA kit, which facilitates a precise quantification of Cry1Ab or Cry1Ac, expressed in transgenic plants.
2. The kit is simple, cost effective and very reliable.
3. It takes about 2hrs for completion of one set of ELISA assay.
4. Each ELISA plate can be used for 96 samples (including four wells for standards and two for blank). Depending on the capabilities of a laboratory, hundreds of samples can be processed in a single day.
5. ELISA plate reader is a requirement for use of the kit.
6. Additionally the ELISA kit can also be used for the quantification of Bt-toxins in Bt(Bacillus thuringiensis) insecticide formulations. Separate standards will be provided on request.

Bt-Express



Bt-Express

1. This is a dipstick format and can be used by even a layman.
2. Bt-Express has been designed for instiantaneous detection of Bt- Toxin in either seeds or plant tissues.
3. It takes about 10 minutes for the test to be completed.
4. The test can be used in fields and does not require any additional facilities for use.
5. All material required for the testing is provided with the kit.
6. The kit is rapid, reliable and ready to use.

Is there field resistance to Bt?

(field collections from around the country)

	LC50 µgCry1Ac/ml diet
2001 Baseline*	0.01 - 0.67
2003-4	0.04 - 0.38
Chinese baseline+	0.091 - 9.093

- Regional variability in resistance within India
  2001-2 67 fold
  2002-3 32 fold
  2003-4 10 fold

- Not yet

**(Kranthi et al 2001)*
+(Wu et al 1999)

Inheritance of Cry 1Ac resistance

Bioassay of 2 resistant strains (RF 93 and 205) selected from Bt field survivors and susceptible strain using semi-synthetic diet and Bt plants (Mech 184-Bt).

Inheritance of Cry1Ac resistance in *H. armigera*

♀ x ♂	n	LD50	95%FL	SLOPE+ SE	RF	D	D_{LC}	ηE
RES-Ac (R)	120	3.35	1.42-17.70	0.8 ± 0.2	93			
SUS-G (S)	144	0.036	0.02-0.06	1.8 ± 0.3				
SxR	360	0.24	0.17-0.34	1.1 ± 0.1		-0.16	0.42	0.53
RxS	120	0.25	0.15-0.42	1.2 ± 0.2		-0.14	0.43	
(RS)xR	120	1.30	0.65-3.20	0.9 ± 0.1				
(SR)xS	120	0.19	0.09-0.36	0.9 ± 0.1				
RES-Bt (R)	240	15.02	10.0-26.7	1.4 ± 0.2	205			
SUS-G (S)	268	0.073	0.05-0.10	2.2 ± 0.3				
SxR	180	1.53	0.6-5.3	0.9 ± 0.1		0.14	0.57	1.11
RxS	144	1.35	0.78-2.48	1.0 ± 0.1		0.09	0.54	
(SR)xR	120	3.96	2.0-8.89	1.0 ± 0.2				
Rx(SR)	120	5.53	2.3-20.66	1.0 ± 0.2				
Rx(RS)	120	5.58	2.2-29.0	0.9 ± 0.2				
(RS)xR	160	2.58	1.3-6.22	0.8 ± 0.1				
Sx(SR)	120	0.89	0.18-3.00	1.1 ± 0.2				
(SR)xS	144	1.07	0.62-1.92	1.0 ± 0.1				
Sx(RS)	120	1.16	0.5-2.58	1.1 ± 0.2				
(RS)xS	144	1.29	0.72-2.16	1.2 ± 0.2				

- Autosomal
- Monogenic
- Semi-dominant

Implications of resistance inheritance

- Survival on Mech 184 - Bt plants (75-85 days old)

  RR 75%

  RS 33%

  SS 5% (susceptible strain)

- Semi-dominance
  - Also applies to effective dominance on plants
  - Undermines the high dose strategy

Note – Australian H.armigera resistance is incompletely recessive (0.26)

Resistance gene frequency

(using F2 screens on field material)

	Iso-female lines	*Frequency of resistance alleles*
North	180	0.0013
Centre	195	0.0025
South	210	0.0023

- At least one resistance allele in each group
- Frequency is worryingly high for a semi-dominant resistance

Better bioassay systems

- Inhibition Concentration 99

  concentration which prevents 99% of larvae from reaching 3rd instar in 5 days 0.091 – 0.109 µg/ml

- Bt seed flour-based diet

  160g Bt seed in 1.3L diet is equivalent to 0.2 Cry 1Ac/ml

  Advantages: Seeds have a very stable toxin content

  Seeds keep for >2yrs

  Bt Cotton has Cry1Ac **and** 1Ab

  MPVII has inly 20% Cry1Ac and is not available

Mechanisms

- *Chinese H.armigera* – cadherin truncation (Wu et al 2004)
- *Australian H.armigera* – not cadherin mediated
- *Indian H.armigera* – suggestions of an aminopeptidase involvement

  - Binding affinity reduced in 6 fold in trypsin activated toxins and 10 fold in protease activated toxins
  - Major portion of APN-1 cDNA of Cry1Ac resistant strain sequenced
    - 16 base substitutions, 6 additions in 2766 nucleotides leading to 17 a.a. differences
    - One of the these is related to glycosylation (Ser^{911} to Phe^{911})

Refugia

Current requirement 20% sprayed border

- 400m^2 replicated blocks.
- 1 larva per plant at 75 and 85 days after sowing.

	Yield sig. diff
Pure Bt	**a**
19:1 rows and mixes	**a,b**
9:1 " " "	**b**
4:1 " " "	**c**

- Recovery: 82-93% on non-Bt
  2-10% on Bt
- Plant to plant movement: little (hybrid spacing)
- Survival on Bt flowers and bolls: some extra
- Resistance in survivors: no enhancement detected

‘BT-Adapt’ - Modelling the course of resistance development

- Simulation model - genetic and ecological parameters

Genetic	**Ecological**	**Control**
Dominance	District cropping pattern	Efficacy in Bt crop
RRsurvival on Bt	Emmigration per generation	Efficacy in non-Bt
SS survival on Bt	Oviposition preferences	
	Natural egg-larval mort.	
	Natural pupal-adult mort.	

- Order of importance of parameters in time to field resistance

1. Relative survival rate for SS, RS and RR (fitness)
2. Proportion of area under Bt
3. Dominance of resistance allele
4. Initial frequency of resistance gene

Time to 0.5 resistance allele frequency

% Bt cotton	No additional pest management	50 % control in non-Bt only	50% in non-Bt 90% in Bt
40	11	7	23
30	16	10	32
20	25	13	45
10	51	31	70

- Emphasises the importance of additional mortality of survivors in the Bt crop – parasites, HaNPV, pesticides etc.

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