Study No.11:

Title	**: Pollen flow studies**
Organization	**: Metahelix Life Sciences Private Limited, Bangalore**
Status	**: Year I – Kharif-2005; Completed**
	Year II – Rabi-2006/2007; Completed

Year I study:

Extracted from pages 112-114 of the report submitted by Metahelix Life Sciences of the Strip Trial conducted with *B.t.* and non-*B.t.* cotton carrying Metahelix *cry1C* gene event MLS9124 to RCGM in its meeting on 2 May 2006

a) Objectives

The main objectives of this study were to

(i) Detect the pollen flow from trial field to border row plants

(ii) Estimate the distance of pollen flow from the trial field

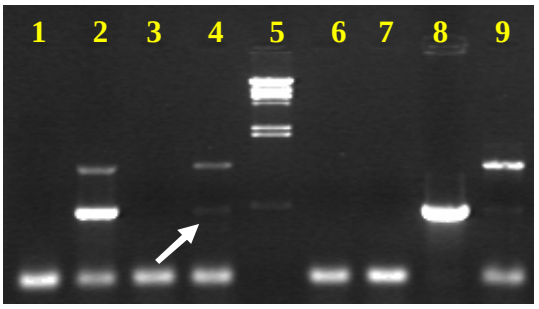
b) Materials and Methods:

The transgenic pollen source was sown in four test plots. Non B.t. plants were sown in 5 rows (refered as border rows) at a distance of 1 m from the test plots. Five border rows were planted with a row spacing of 90 cm. Two bolls were collected from each border row from all test plots randomly. The sampling for the bolls were done between 90-105 days after sowing (DAS). Uniformity of boll development stage was ensured while sampling between the trial plot plants and the border row plants was done. Polymerase chain reaction was carried out to detect the presence of gene in border row plants using the DNA isolated from the pooled seeds.

c) Results and conclusions:

Fig. 1

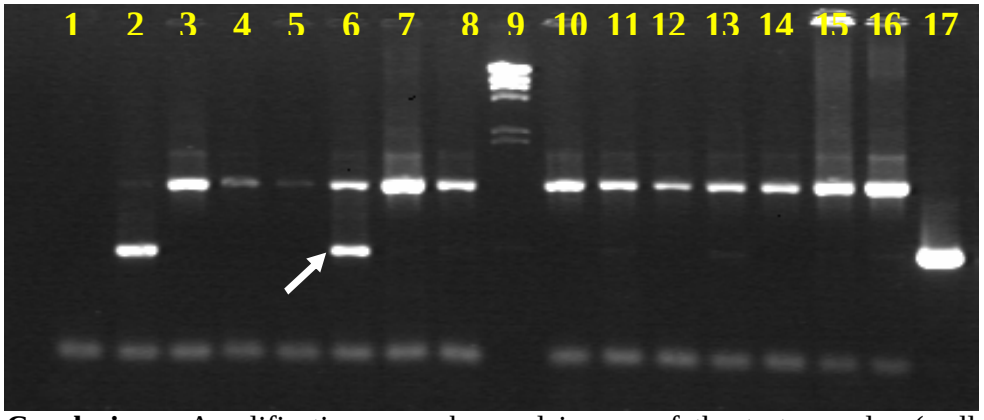
1. H_2O control
2. Transgenic DNA - positive control
3. Plant from 5th border row, Plot 1
4. Plant from 2nd border row, Plot 1
5. ?dIII Marker
6. Plant from 1st border row, Plot 1
7. Plant from 4th border row, Plot 1
8. pMH 87
9. Non-*B.t.* parent- (negative control)



Conclusions: Amplification was observed in one sample (well no. 4) i.e. Plant from 2nd border row, Plot 1, indicating the pollen flow from the transgenic parent till the 2nd border row.

Fig. 2:

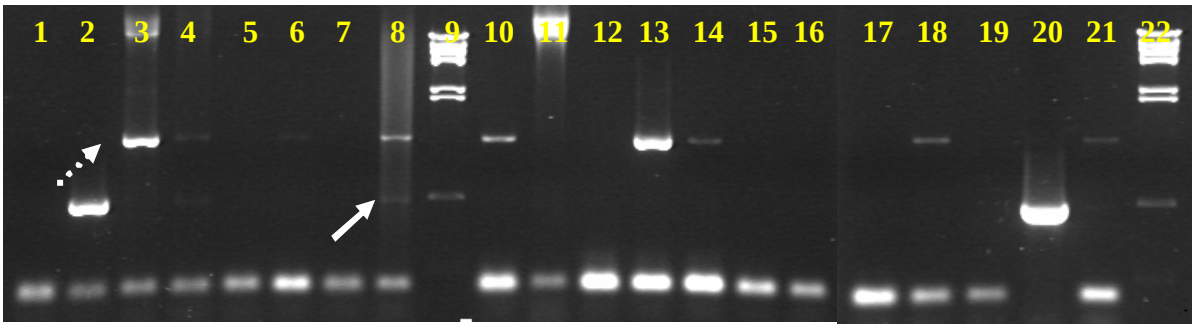
1. H_2O control
2. Transgenic (+ve control)
3. Plant from 3rd border row, Plot 3
4. Plant from 1st border row, Plot 3
5. Plant from 3rd border row, Plot 4
6. Plant from 2nd border row, Plot 1
7. Plant from 4th border row, Plot 3
8. Plant from 3rd border row, Plot 1
9. ?dIII Marker
10. Plant from 1st border row, Plot 4
11. Plant from 5th border row, Plot 4
12. Plant from 3rd border row, Plot 4
14. Plant from 1st border row, Plot 2
15. Plant from 4th border row, Plot 4
16. Plant from 2nd border row, Plot 2
17. pMH 87



Conclusions: Amplification was observed in one of the test samples (well no. 6) i.e. Plant from 2nd border row, Plot , indicating the pollen flow from the trial plot till the 2nd border row.

Fig. 3

1. H_2O control
2. Transgenic
3. Plant from 2nd border row, Plot 1
4. Plant from 1st border row, Plot 1
5. Plant from 1st border row, Plot 2
6. Plant from 3rd border row, Plot 2
7. Plant from 2nd border row, Plot 2
8. Plant from 2nd border row, Plot 4
9. ?dIII Marker
10. Plant from 5th border row, Plot 3
11. Plant from 3rd border row, Plot 4
12. Plant from 2nd border row, Plot 38
13. Plant from 3rd border row, Plot 1
14. Plant from 1st border row, Plot 16
15. Plant from 3rd border row, Plot 3
16. Plant from 1st border row, Plot 3
17. Plant from 5th border row, Plot 4
18. Plant from 2nd border row, Plot 1
19. Plant from 4th border row, Plot 4
20. pMH 87
21. Non-*B.t.* parent
22. ?dIII Marker



Conclusions: Amplification was observed in one sample (well number 8) i.e. Plant from 2nd border row, Plot 4.

Fig 1,2 and 3. Expected band size: Internal control band (GhrbcS): 1.1 kb (dotted arrow); *cry1C* specific band: 500 bp. Marker used is HindIII ? . Cry1C gene specific band in the test plants is indicated by white arrow

410

Table 1: Distance of pollen flow from the transgenic to non transgenic cotton

details	plot no.	Image-well #	results	distance from transgenic source
5th Border row	Plot 1	image 1-3	-	4.6 m
2nd Border row	Plot 1	image 1-4	+	1.9 m
1st Border row	Plot 1	image 1-6	-	1.0 m
4th Border row	Plot 1	image 1-7	-	3.7 m
3rd Border row	Plot 3	image 2-3	-	2.8 m
1st Border row	Plot 3	image 2-4	-	1.0 m
3rd Border row	Plot 4	image 2-5	-	2.8 m
2nd Border row	Plot 1	image 2-6	+	1.9 m
4th Border row	Plot 3	image 2-7	-	3.7 m
3rd Border row	Plot 1	image 2-8	-	2.8 m
1st Border row	Plot 4	image 2-10	-	1.0 m
5th Border row	Plot 4	image 2-11	-	4.6 m
3rd Border row	Plot 4	image 2-12	-	2.8 m
1st Border row	Plot 2	image 2-13	-	1.0 m
4th Border row	Plot 4	image 2-14	-	3.7 m
2nd Border row	Plot 2	image 2-15	-	1.9 m
2nd Border row	Plot 1	image 3-3	-	1.9 m
1st Border row	Plot 1	image 3-4	-	1.0 m
1st Border row	Plot 2	image 3-5	-	1.0 m
3rd Border row	Plot 2	image 3-6	-	2.8 m
2nd Border row	Plot 2	image 3-7	-	1.9 m
2nd Border row	Plot 4	image 3-8	+	1.9 m
5th Border row	Plot 3	image 3-10	-	4.6 m
3rd Border row	Plot 4	image 3-11	-	2.8 m
2nd Border row	plot 38	image 3-12	-	1.9 m
3rd Border row	Plot 1	image 3-13	-	2.8 m
1st Border row	plot 16	image 3-14	-	1.0 m
3rd Border row	Plot 3	image 3-15	-	2.8 m
1st Border row	Plot 3	image 3-16	-	1.0 m
5th Border row	Plot 4	image 3-17	-	4.6 m
2nd Border row	Plot 1	image 3-18	-	1.9 m
4th Border row	Plot 4	image 3-19	-	3.7 m

Conclusion: There is gene flow from the transgenic trial plot to the non-transgenic border plants which is evident from the gene specific amplification in the border row plant DNA The flow of pollen is limited up to 1.9 m (Table 1), from the respective transgenic plot, indicating the limited pollen movement under the conditions of the field trials, sampling and analysis.

Year II Study:

a. Objective:

To assess the extent of flow of pollen from transgenic cotton (*Gossypium hirsutum*) expressing cry1C gene into non transgenic cotton (*G. hirsutum*).

b. Materials & Methods

The experimental material comprised B.t. cotton variety MHCO0001*B.t.* carrying Metahelix transgenic *cry1C* gene event MLS9124 and non-transgenic Metahelix commercial cotton hybrid "Badri". The experimental field was laid out with the MHCO0001*B.t.* plants in the centre and the non-*B.t.* cotton plants, Badri, were sown in concentric squares at regular intervals of 1 meter extending up to 48 meters, surrounding the transgenic source plot. The transgenic MHCO0001*B.t.* cotton plants were sown in a plot of 8.64 sq. m. A spacing of 60 x 60 cm^2 was maintained between the plants. The total field area was 2510 sq.m and the sowing was done on 15th of January, 2007 at Metahelix R & D farm, Bangalore. (Fig. 2)

At peak flowering, opened flowers were tagged everyday in transgenic and non-transgenic plants. The tagging of flowers was continued everyday for 20 days. This was done to ensure that sufficient sampling was done to collect bolls developed from flowers which were open at the same time in both transgenic and non-transgenic genotypes. During this period, normal insect activity was observed.

At boll bursting stage, only the bolls developed from the tagged flowers were harvested and bulked according to date of flowering and distance from *B.t.* plot. The seeds from such collected bulks were used for DNA extraction and for PCR confirmation for the presence of the transgene *cry1C*. Two sets of primers were used, one to detect the native albumin gene and the other for *cry1C*.

c. Results and Conclusion:

The seed samples collected from the non transgenic cotton plants surrounding the transgenic *B.t.* cotton were tested for the presence of the *cry1C* gene using multiplex PCR. None of the non transgenic cotton showed *cry1C* gene indicating complete absence of gene flow. Though our earlier study shows sporadic pollen flow to second row, the present study showed no pollen flow even to the plants at 1 m distance.

Fig 1a: PCR results of the samples analyzed for pollen flow to non transgenic cotton

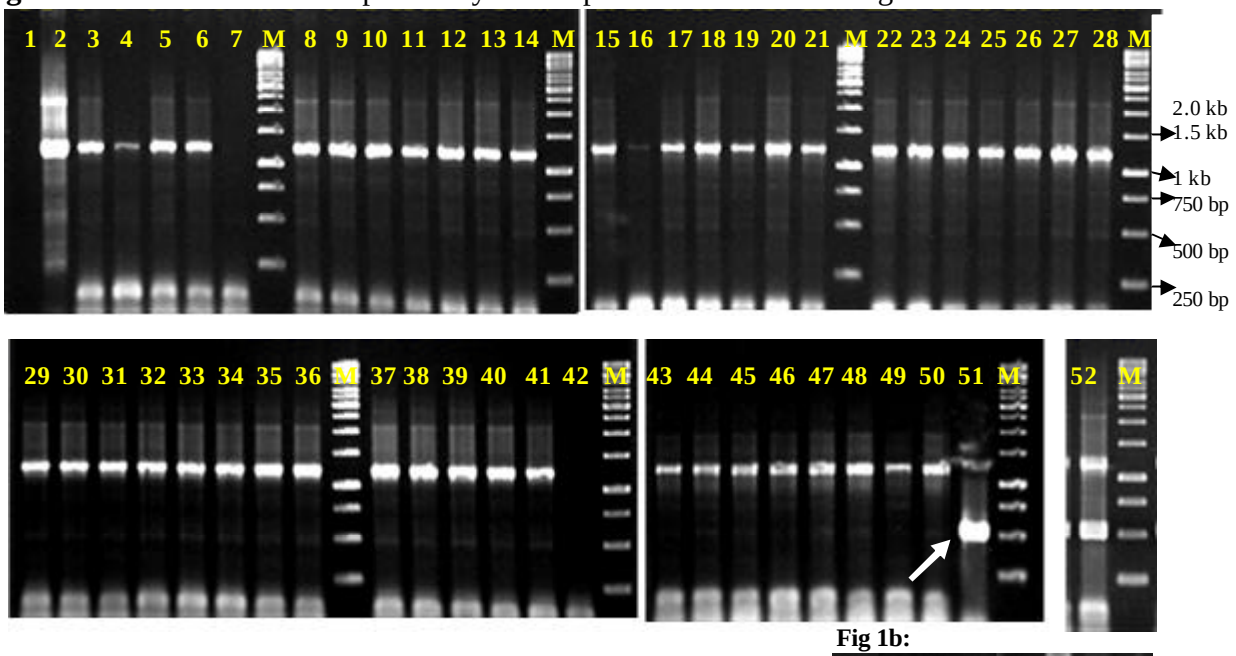
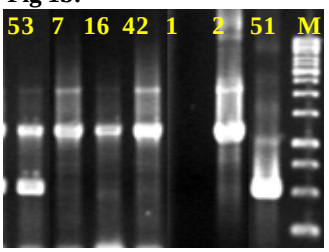


Fig 1a: Expected band size: Internal control band (2S albumin): 1.1 kb; *cry1C* specific band: 500 bp. Marker used is GeneRuler (#SMO311) 1Kb DNA Ladder from Fermentas.
Cry1C gene specific band is seen in lanes 51 (indicated by arrow) and 52, respectively the plasmid with the gene construct and the transgenic plant DNA.. Samples corresponding to Lanes 7, 16 and 42 were repeated and the image is given on the Figure 1b.

Fig 1b:



Lane number and details:

Lane no.	Details	Lane no.	Details	Lane no.	Details	Lane no.	Details	Lane no.	Details
1	water	11	9 M	21	19 M	31	29 M	41	39 M
2	Non-Tr cotton[a]	12	10 M	22	20 M	32	30 M	42	40 M
3	1 M[b]	13	11 M	23	21 M	33	31 M	43	41 M
4	2 M	14	12 M	24	22 M	34	32 M	44	42 M
5	3 M	15	13 M	25	23 M	35	33 M	45	43 M
6	4 M	16	14 M	26	24 M	36	34 M	46	44 M
7	5 M	17	15 M	27	25 M	37	35 M	47	45 M
8	6 M	18	16 M	28	26 M	38	36 M	48	46 M
9	7 M	19	17 M	29	27 M	39	37 M	49	47 M
10	8 M	20	18 M	30	28 M	40	38 M	50	48 M
51	pMH plasmid	52	Transgenic cotton						

[a]: DNA from non-transgenic cotton

[b]: M indicates the metre at which the samples were collected for PCR analysis. Lane 3 to 50: Samples collected from 1 to 48 m Lane 51: The plasmid containing the construct was used as a positive control. Lane: 52: DNA isolated from the transgenic cotton.

Fig. 2: Field picture of the Trial, (Crop age 30 days) (Rectangle marked in black indicates source *B.t.* plants)