

## **ADDENDUM 3**

### **Biochemical Analysis of *B.t.* cotton seeds (Carrying *cryIC* gene, event MLS9124)**

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**Title** : Biochemical Analysis of *B.t.* cotton seeds (Carrying *cryIC* gene, event MLS9124)  
**Organization** : Metahelix Life Sciences Private Limited, Bangalore  
**Status** : Completed

**Objective:**

The objective of this study was to estimate the biochemical composition (major and minor elements) of seeds from transgenic *B.t.* cotton containing *cryIC* gene and the non *B.t.* cotton.

**Introduction:**

The cotton seeds from both *B.t.* and the non *B.t.* plants were analyzed by the University of Agricultural Sciences, Bangalore and Dharwad, Karnataka.

**Results and Conclusions (Part 1)**

The following were the composition of the cotton seeds of *B.t.* and non *B.t.* analyzed by UAS, Bangalore.

No	Parameter	Sample I ( <i>B.t.</i> )	Sample II (Non- <i>B.t.</i> )
1	N (%)	3.13	4.05
2	P <sub>2</sub> O <sub>5</sub> (%)	1.69	1.98
3	K <sub>2</sub> O (%)	1.04	1.51
4	Ca (%)	2.26	2.07
5	Mg (%)	1.02	0.91
6	Fe (ppm)	54.0	48.5
7	Mn (ppm)	28.6	31.5
8	Zn (ppm)	46.0	46.5
9	Cu (ppm)	25.5	27.8
10	Oil content	22.8	18.3

The parameters tested were comparable between the *B.t.* and the non *B.t.* samples and can be concluded that the transgene has not altered the biochemical composition of the cotton seeds.

**UNIVERSITY OF AGRICULTURAL SCIENCES,**

GKVK, Bangalore - 560 065,

SS&AC/ 369 /07-08

21<sup>st</sup> JANUARY 2008

To,  
Dr. M.J. Vasudev Rao  
President Ag Technologies,  
Metahelix Life Science Private Ltd  
Plot No.3, KIADB 4<sup>th</sup> Phase  
Bommasandra  
Bangalore-79

Through Director of Research, UAS, Bangalore.

Sir,

Sub: Analytical results of cotton seed samples ...reg

Ref: DR/TT- 155 /2007-08 dt 27-12-2007.

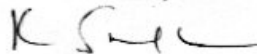
Please find here in below the Analytical results of cotton seed samples sent by you for analysis in the Dept. of Soil Science and Agricultural Chemistry, Agricultural College, GKVK, Bangalore-65

Parameters	cotton seeds samples	
	I	II
N (%)	3.13	4.05
P <sub>2</sub> O <sub>5</sub> (%)	1.69	1.98
K <sub>2</sub> O (%)	1.04	1.51
Ca (%)	2.26	2.07
Mg (%)	1.02	0.91
Fe (ppm)	54.0	48.5
Mn (ppm)	28.6	31.5
Zn (ppm)	46.0	46.5
Cu (ppm)	25.5	27.8
Oil Content (%)	22.8	18.3

DRTT-155/2007-08 dt: 22-01-2008

The result should not be utilized for legal / commercial purposes without prior consent of this Directorate.

Yours faithfully



Professor & Head

Dept. of Soil Science & Agricultural Chemistry

*Handwritten signature and date 21/1/08*

College of Agriculture, G.K.V.S.  
Bangalore - 560 065

Forwarded.

*Handwritten signature of the Director of Research*  
Director of Research

Director of Research

University of Agril. Sciences

GKVK Campus, Bangalore-560 065


## Results and Conclusions (Part II)

The following were the composition of the fatty acid profile (percentage) of cotton seeds of *B.t.* and non *B.t.* analyzed by UAS, Dharwad

### FATTY ACID PROFILE IN COTTON SAMPLES

SL. NO.	SAMPLE	PALMITIC	STERAIC	OLEIC	LINOLEIC	ARACHIDIC
1	A	23.20	2.03	17.51	57.05	0.20
2	A	23.13	1.74	17.16	57.74	0.20
Mean		23.16	1.88	17.33	57.40	0.20
3	B	23.02	1.56	16.92	58.28	0.20
4	B	22.68	1.32	17.05	58.73	0.20
Mean		22.85	1.44	16.98	58.50	0.20

The parameters are in percentage

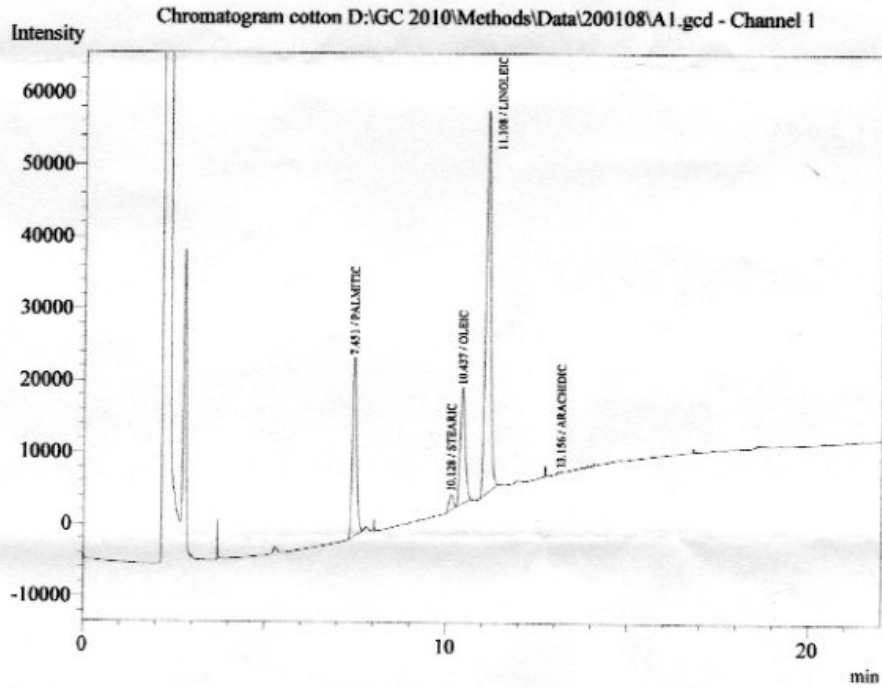
  
Special Officer (Seed  
University of Agri. Sciences  
Dharwad.

The parameters tested were comparable between the *B.t.* and the non *B.t.* samples and can be concluded that the transgene has not altered the biochemical composition of the cotton seeds.

# **UAS DHARWAD SEED UNIT**


## Sample Information **A**

Analysis Date & Time: 1/20/2008 12:25:13 PM  
 Sample Name : cotton  
 Sample ID : 2  
 Data Name : D:\GC 2010\Methods\Data\200108\A1.gcd  
 Method Name : D:\GC 2010\Methods\FAME 220607.gcm



Peak Table - Channel 1

Peak#	Name	Ret.Time	Conc. %	Area %	Height %
1	PALMITIC	7.451	23.207	201397	24769
2	STEARIC	10.128	2.035	17659	2156
3	OLEIC	10.437	17.510	151960	16123
4	LINOLEIC	11.108	57.053	495122	53137
5	ARACHIDIC	13.156	0.195	1689	186
Total				867827	96371

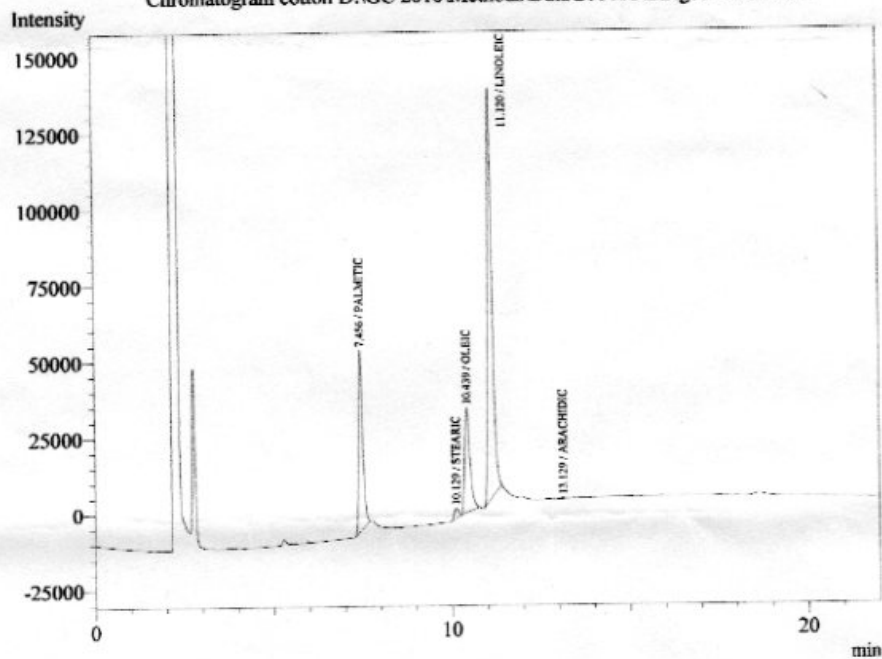
  
 Special Officer (Seed)  
 University of Agril. Sciences  
 Dharwad.

# **UAS DHARWAD SEED UNIT**

## Sample Information **B**


Analysis Date & Time: 1/20/2008 2:32:51 PM  
 Sample Name : cotton  
 Sample ID : 7  
 Data Name : D:\GC 2010\Methods\Data\200108\BB.gcd  
 Method Name : D:\GC 2010\Methods\FAME 220607.gcm

Chromatogram cotton D:\GC 2010\Methods\Data\200108\BB.gcd - Channel 1



Peak Table - Channel 1

Peak#	Name	Ret. Time	Conc.	Area%	Height%
1	PALMITIC	7.456	22.680	22.6802	25.5865
2	STEARIC	10.129	1.326	1.3261	1.4554
3	OLEIC	10.439	17.052	17.0516	14.8321
4	LINOLEIC	11.120	58.740	58.7395	57.9575
5	ARACHIDIC	13.129	0.203	0.2026	0.1685
Total				100.0000	100.0000

  
 Special Officer (Seed)  
 University of Agril. Sciences  
 Dharwad.

### PCR and ELISA CONFIRMATION OF *B.T.* & NON *B.T.* COTTON SEED

**Objective:** Quality control of the seed material from Cry1C-9124 intra hirsutum cotton hybrids to be used for the biosafety studies at NDRI, Karnal and baseline susceptibility studies conducted at Metahelix Life Sciences Pvt. Ltd., Bangalore.

1. Confirmation of the transgenic nature by PCR based testing
2. Confirmation of presence of protein by ELISA and Quantification of Cry1C protein in the seed material.

#### 1. PCR confirmation:

PCR confirmation was done using the following primers and conditions:

##### **Primers Used**

Internal Control: (1) Primer 229 Gh 2S alb U and (2) Primer 230 Gh 2S alb L

Cry1C Specific: (1) Primer 117 MH1CGh2-U and (2) Primer 118 MH1CGh2-L

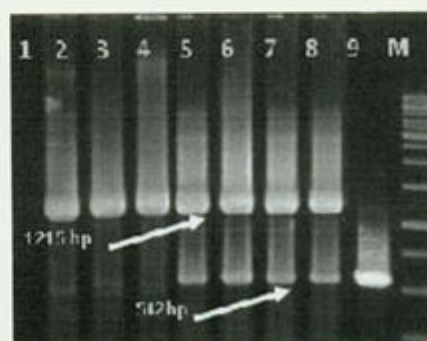
##### **PCR Conditions: (Eppendorf master Cycler)**

Step	Temp°C	Duration	No. of cycles
1	94	2 min	1
2	94	15 sec	
3	60	20 sec	Minus 0.5 deg for 10 cycles
4	72	1 min	
5	94	15 sec	
6	55	20 sec	30 cycles
7	72	1 min	
8	72	5 min	1
9	END		

**Expected Band Sizes:** 1215 (internal band) & 542 bp (Cry1C specific band)

##### **Legend**

1. Water control
2. Non transgenic leaf DNA (-ve)
3. Non *B.t.* seed DNA 1
4. Non *B.t.* seed DNA 2
5. *B.t.* seed DNA 1
6. *B.t.* seed DNA 2
7. Transgenic cotton leaf DNA 1
8. Transgenic cotton leaf DNA 2
9. Plasmid cry1c



**Conclusion:** The expected 542 bp amplicon has been observed in the transgenic seed powder DNA only, 1215 bp cotton internal control amplicon was observed in all the cotton DNA samples, as expected water and negative controls were clear.



## **2. ELISA confirmation and Quantification**

Confirmation and quantification of Cry1C protein was done using the Quantiplate kit for Cry1C (Envirologix, USA, Catalog number AP 007)

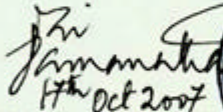
No.	Sample	Concentration
1	Blank	NA
2	Std 1 ppb	1.20 ng /ml
3	Std 5 ppb	5.17 ng/ml
4	Std 10 ppb	9.71 ng /ml
5	B.t. -5 X diluted	2.32 µg/g
6	B.t. 10X diluted	2.55 µg / g
7	Non B.t. 5X diluted	NA

\*All blank reduction values

**Result:** The absorbance value observed for the Non B.t. cotton seed sample was similar to the blank values and the colour development was not seen. Blue colour development was seen in transgenic samples, which was clearly absent in negative controls and non transgenic cotton seed sample. The average amount of Cry1C protein in the seed samples was 2.44 µg / g of seed powder.

Declaration:

I hereby declare that the certificate of quality presented in the above results are true to my knowledge and is made on the basis of experiments conducted at our facility

  
17th Oct 2007  
(Vai. Ramanathan)  
Head- Genomics





# UNIVERSITY OF AGRICULTURAL SCIENCES

Directorate of Research, Krishinagar, Dharwad- 580 005(Karnataka)

Dr. R. R. Hanchinal



Phone : 0836-2745903 / / 2740291

Fax : 0091-0836-2748377 / 2 448349

Email : druasd@sancharnet.in

[druasd@rediffmail.com](mailto:druasd@rediffmail.com)

Director of Research

No.DR/ Metahelix/ <sup>2253</sup> /2007-08 <sup>5301A</sup>

Date: 24.01.2008

To,

✓ Dr. M.J. Vasudeva Rao  
President Ag Technologies  
Metahelix Life Sciences Pvt. Ltd.  
Plot No. 3, KIADB 4<sup>th</sup> Phase,  
Bommasandra,  
Bangalore – 560 099  
Fax : 08110415074

Sir,

**Sub: Forwarding of Chemical testing report....reg.**


In response to your letter No. Nil dated 19.12.2007, the results of analysis of Cotton seed sample for Gossypol content are as follows :

Sample A - 0.182%

Sample B - 0.160%

This is for your kind information.

Yours faithfully,

  
Director of Research  
U.A.S. Dharwad-5  
U.A.S. Dharwad-5

Encl: As above