

भारत सरकार  
विज्ञान और प्रौद्योगिकी मंत्रालय  
बायोटेक्नोलॉजी विभाग  
GOVERNMENT OF INDIA  
MINISTRY OF SCIENCE & TECHNOLOGY  
DEPARTMENT OF BIOTECHNOLOGY



ब्लॉक-2, 7 वां तल, सी० जी० ओ० कम्प्लेक्स  
लोदी रोड, नई दिल्ली-110003  
Block-2, 7th Floor C.G.O. Complex  
Lodi Road, New Delhi-110003

①

No. BT/BS/17/02/94-PID

Dated : 01.12.2005.

To

M/s. Maharashtra Hybrid Seeds Company Ltd,  
Resham Bhawan, 4<sup>th</sup> Floor,  
78, Veer Nariman Road, Mumbai - 400 020.

Subject: Application submitted for approval to carry out "studies on estimation of the alkaloid contents in Bt. Brinjal in comparison with its non-Bt. counterparts" at Indian Institute of Chemical Technology, Hyderabad.

Gentlemen,

The Department is to refer to your letter No. SBD/1267/2005 dated 19.09.2005 on the above mentioned subject and to inform you that the application was considered by the RCGM in its 35<sup>th</sup> meeting held on 27.10.2005. While approving the protocol for estimation of the alkaloid contents in Bt. Brinjal in comparison with its non-Bt. counterparts at Indian Institute of Chemical Technology, Hyderabad, RCGM directed that the samples should be drawn and used from the same age group of the fruits from non-Bt and Bt brinjal plants.

Kindly acknowledge the receipt of the letter.

Yours faithfully,

(T.V. Ramanaiah)  
Member Secretary, RCGM &  
Scientist-F, DBT

## Details of the proposed experiment

### **Comparison of alkaloid contents of Bt brinjal containing *cryI*Ac gene and its non-Bt counterpart.**

The following work will be carried out at Indian Institute of Chemical Technology (IICT), Hyderabad towards comparative studies of Bt brinjal and its non-Bt counterpart.

1. Isolation of marker/major chemical constituent by the following techniques.
  - a). Extraction of the dried plant material following usual extraction techniques.
  - b). Chromatographic separation of the extract for isolating the major chemical constituent/alkaloid will be undertaken. So far in the literature only one alkaloid (Calystegine B2) is known to be present in brinjal (*Solanum melongena*) (Ducrot, P.-H. et al., Tet. Lett., 1990, 31, 3879; 3883).

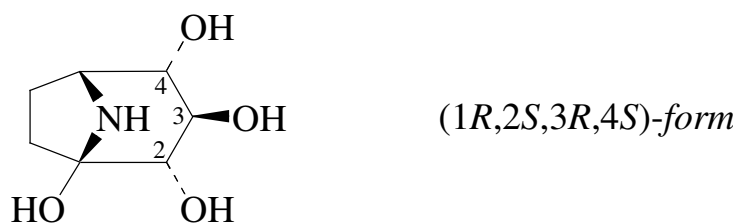


Figure 1: Molecular representation of Calystegine B2

2. Development of analytical methodology

An analytical method will be developed for the quantification of the major alkaloid/chemical constituent in the raw material using HPTLC/HPLC.
3. Comparison of chemical finger prints of Bt brinjal containing *cryI*Ac gene and its non-Bt counterpart

The amount of the marker/major chemical constituent will be estimated in both the samples using the above analytical method. Chemical fingerprints of both Bt brinjal and its non-Bt counter part will be generated and comparative studies will be undertaken.

## Summary Results

From the *Solanum melongena* dry fruit and root powder samples provided by M/S Mahyco, two alkaloids namely, solamargine and solasonine were isolated. The method developed enables direct identification and determination of solamargine and solasonine the two alkaloids present in the given samples of *S. melongena* fruit and root powders.

### **% Abundance of solamargine in non-BT fruit powder.**

Concentration of crude extract: 1.0mg/ml

From Calibration curve the amount of solamargine present : 0.06077mg/ml

100mg of crude extract contains 6.07mg of solamargine

1.12 gm of alkaloid extract contains 67.984 mg of solamargine

1.12 gm of alkaloid extract obtained from 250gm of non BT fruit powder

250gm of plant material contains 67.985 mg of solamargine

Abundance of solamargine in plant material is 0.0272%

### **% Abundance of solamargine in BT fruit powder.**

Concentration of crude extract: 1 mg/ml

From Calibration curve the amount of solamargine present : 0.0374mg/ml

100mg of crude extract contains 3.74mg of solamargine

1.4 g of alkaloid extract contains 52.36 mg of solamargine

1.40 g of alkaloid extract obtained from 250gm of BT fruit powder

250g of plant material contains 52.36 mg of solamargine

Abundance of solamargine in plant material is 0.0209%

**% Abundance of solamargine in BT root powder.**

Concentration of crude extract: 1.5mg/ml

From Calibration curve the amount of solamargine present : 0.387mg/ml

100mg of crude extract contains 25.8mg of solamargine

0.169 g of alkaloid extract contains 43.6 mg of solamargine

0.169g of alkaloid extract obtained from 100g of BT root powder

Abundance of solamargine in plant material is 0.0436%

**% Abundance of solamargine in non-BT root powder.**

Concentration of crude extract: 1.0mg/ml

From Calibration curve the amount of solamargine present : 0.669mg/ml

100mg of crude extract contains 66.9mg of solamargine

0.428 g of alkaloid extract contains 66.9 mg of solamargine

0.428 g of alkaloid extract obtained from 100g of non-BT root powder

Abundance of solamargine in plant material is 0.0669 % .

**% Abundance of solasonine in BT fruit powder.**

Concentration of crude extract: 1.35mg/ml

From Calibration curve the amount of solasonine present : 0.071mg/ml

100mg of crude extract contains 5.25 mg of solasonine

1.40 g of alkaloid extract contains 73.5 mg of solasonine

1.40g of alkaloid extract obtained from 250g of BT fruit powder

Abundance of solasonine in plant material is 0.029%

**% Abundance of solasonine in non-BT fruit powder.**

Concentration of crude extract: 1.0mg/ml

From Calibration curve the amount of solasonine present : 0.057mg/ml

100mg of crude extract contains 5.7 mg of solasonine

1.120g of crude extract contains 63.8 mg of solasonine

1.120g of alkaloid extract obtained from 250g of non-BT fruit powder

Abundance of solasonine in plant material is 0.0255%

**% Abundance of solasonine in BT root powder.**

Concentration of crude extract: 1.5mg/ml

From Calibration curve the amount of solasonine present : 0.105mg/ml

100mg of crude extract contains 7.0 mg of solasonine

0.428g of crude extract contains 29.96 mg of solasonine

0.428g of alkaloid extract obtained from 100g of BT root powder

Abundance of solasonine in plant material is 0.0299%

**% Abundance of solasonine in non-BT root powder.**

Concentration of crude extract: 1.0mg/ml

From Calibration curve the amount of solasonine present : 0.075mg/ml

100mg of crude extract contains 7.5 mg of solasonine

0.169g of crude extract contains 12.67 mg of solasonine

0.169g of alkaloid extract obtained from 100g of non-BT root powder

Abundance of solasonine in plant material is 0.0126% .