Bhutan Agriculture and Food Regulatory Authority (BAFRA)

Ministry of Agriculture and Forests

Bt-Brinjal surveillance report

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1. Background

During the 5th Annual "South Asia Biosafety Conference" held from September 11-13, 2017 in Bangalore, one of the delegations from Bangladesh verbally submitted that Bt-Brinjal was approved to be used as food or processing and cultivation in 2013. He also pointed that it is grown freely in Bangladesh including places near bordering town of India and as per his knowledge no post-monitoring is been carried out so far. As such it is felt imperative to carry out surveillance on varieties of brinjal available in our market as there is likely that GMO brinjal may come into Bhutan through informal trade. The GMO in viable forms are prohibited by Biosafety Act of Bhutan 2015 and its Rules and Regulations 2018. According to Bangladesh Agricultural Research Institute (BARI), it developed the four Bt-brinjal varieties namely, Bt-Uttara, Bt-Kajla, Bt-Noyontara and Bt-ISD 006 as shown in Table 1 below.

As per the internet search, Bt-brinjal was developed by the Maharashtra Hybrid Seeds Company (Mahyco). The company used a DNA construct containing the *cry1Ac* gene, a CaMV 35S promoter and the selectable marker genes *nptII* and *aad*, to transform young cotyledons of brinjal plants. A single copy elite event, named EE-1, was selected and introduced into hybrid brinjal in Mahyco's breeding program. As per the literature review, cry1Ac is from *Bacillus thuringiensis* subsp. Kurstaki strain HD73, which produces delta-endotoxin and confers resistance to lepidopteran insects by selectively damaging their midgut lining.

Mahyco generously donated the Bt-brinjal technology to the Tamil Nadu Agricultural University (TNAU), Coimbatore and University of Agricultural Sciences (UAS), Dharwad. The event EE-1 was backcrossed into open-pollinated brinjal varieties. Mahyco also donated the technology to public research institutions in the Philippines and Bangladesh. Bt-brinjal was developed to prevent itself from one of the serious pest called fruit and shoot borer (FSB) *Leucinodes orbonalis* and also from other insect pests.

2. Objectives

The objectives of this study are to:

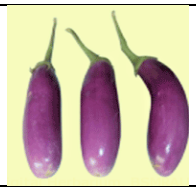
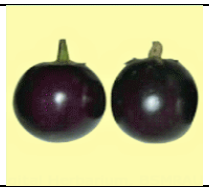
- *Study the presence of Bt-brinjal in vegetable markets/shops in one major town and entry points;*
- *If found positive, further analysis by qPCR at NFTL*
- *In doing so to create awareness on Biosafety legislations among importers*

3. Materials and Methods

3.1. Study design

This survey was conducted in 4 official entry points, one major town and it is carried out by respective BAFRA field officials. The study was carried out to find out if the four varieties of bt-brinjal shown in table 1 are present in the Bhutanese market.

Table 1: Varieties of brinjal

			
Bt-Uttara	**Bt-Kajla**	**Bt-Nayantara**	**Bt-ISD 006**

Pictures shown above were downloaded from internet, as Mahyco, India was not in position to share pictures.

3.2. Sample size and sampling method

Sample size was not estimated as this is a pilot survey. In addition the number of samples tested was determined based on the number of test kits distributed to respective BAFRA offices. Information on test kits is as given below in table 2 and the details of test kits distributed to the respective BAFRA offices is as presented in table 3.

Table 2: Information on test kit

SL.	Supplier/ Manufacturer	Product Description	Pack Size	Price per unit	Quantity	Total
1	Maharashtra Hybrid Seeds Co. Pvt.Ltd.	DGX020 DesiGenXpresstrips Kit For Cry1 Ac (Provided with all required reagents and components)	50 Lateral Flow strips/pack	INR. 3000	5 kits	INR. 15,000 (exclusive of taxes and shipping charges)

Table 3: Distribution of test kit

Sl.#	Name of BAFRA office	Nos. of strip issued
1	Samdrupjongkhar	50
2	Phunstholing	50
3	Samtse	50
4	Thimphu (CFM)	50
5	Gelephu	50

3.3. Sample testing

GMO can be tested either by using rapid test kit or by more advanced techniques such as real time PCR. Since advanced techniques are quite expensive, it is always better and cost effective to have screening of GMO elements at field level. It is understood that the inserted DNA and its corresponding protein are found in all the cells of the plant and as such, the seeds and/or plant tissue material (e.g. leaves) can be used for GMO testing.

For this survey, rapid test kits were used for screening of particular GM element (Cry1Ac), which is present in Bt-brinjal. Maharashtra Hybrid Seeds Co. Pvt. Ltd. (Mahyco), Mumbai, India was contacted to supply lateral flow strips. These test kits are also used by the regulatory bodies like us and also by developers and researchers.

DesiGen *Xpresstrips* are designed to detect the presence of Cry1Ac Bt protein in Brinjal leaf tissue extracts, in a qualitative (presence/absence) manner. Since it is very difficult to get leaf samples from the vegetable market/shop, we have used Brinjal fruits for analysis.

3.4. Test protocol

Sample preparation, testing and interpretation of the results were done as per the manufacturer's protocol as provided below and represented by figure 1;

3.4.1. How the strip works

Plants that contain the Cry1Ac gene express Cry1Ac Bt protein residues in their tissues. DesiGen *Xpresstrips* are lateral flow devices which detect the protein in extracts from plant samples. Each strip has a sample absorbing pad at one end indicated by the word "Sample" and arrows. This end is to be inserted in the tissue extract. The extract moves up the pad into the clear window, and reaches a second absorbent pad at the top of the strip.

3.4.2. Cross reactivity:

The DesiGen *Xpresstrips* does not distinguish between Cry1Ac residues and Cry1Ab residues in plant extracts.

3.4.3. Sample extraction:

Note: Avoid cross-contamination between samples
Adapted (For brinjal fruits extracts): collect reasonable amount of brinjal fruits from vegetable counters and cut into pieces and put into a mcf tube. Add 500µl-sample extraction buffer. Further crush with a pestle for 30 seconds. Place the *Xpresstrip* directly in this.

3.4.4. Running the assay

1. Remove an *Xpresstrip* from its pack and place the sample end (indicated by the arrows) into the tissue extract, taking care that the liquid level should not cross the white line indicated just below the arrow on strip.

2. The liquid will move up the strip into the top absorbent pad. A line should appear in the white window, near the top absorbent pad, indicating that the assay is functioning properly.

3. Wait for 10 minutes before removing the strip from the sample. If a second line is present near the sample pad, the extract is positive for cry1Ac. If no line appears, the sample is negative.

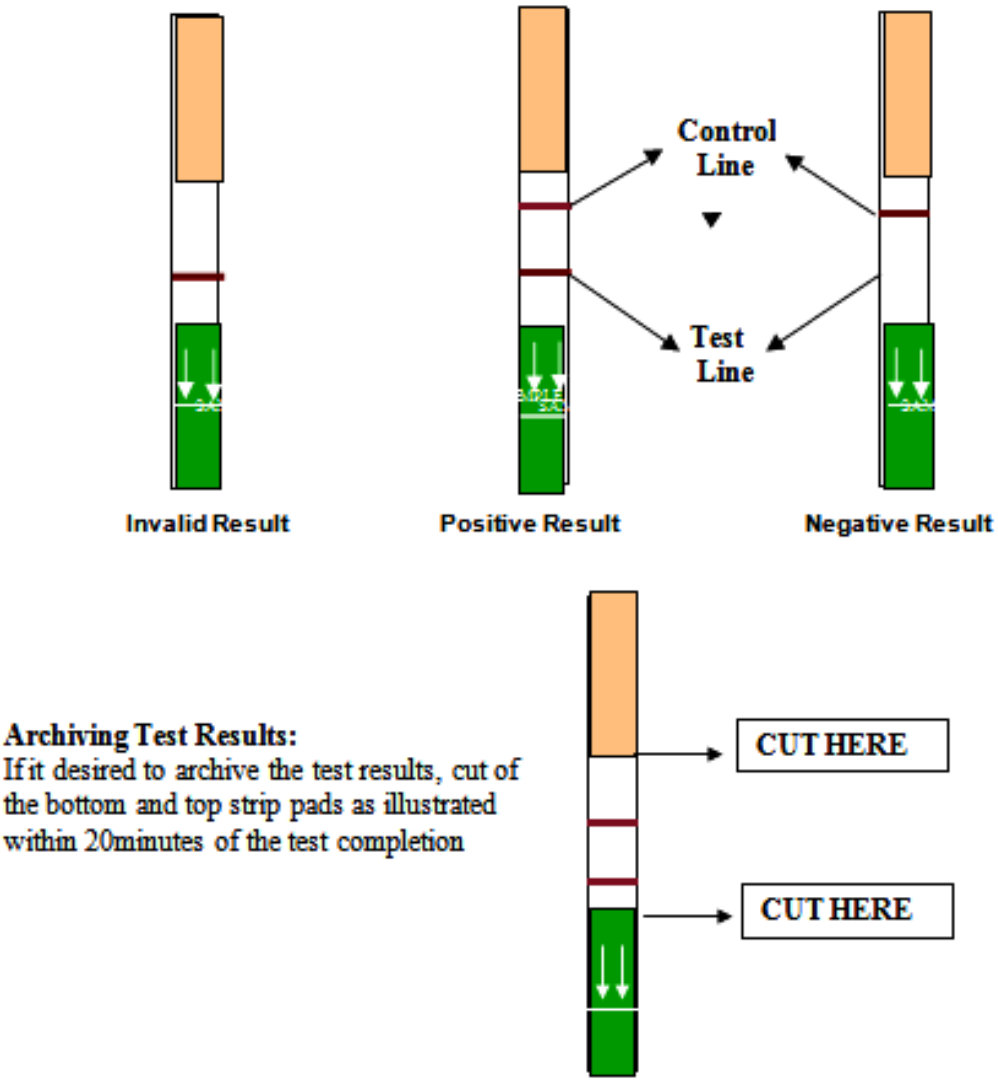


Figure 1: Schematic Representation of Lateral Flow Assay

Note:

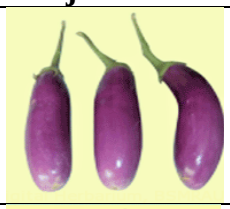
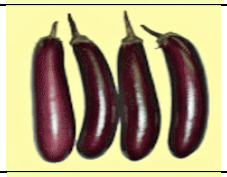
1. Please remove the strips immediately after control line completely appears.
2. The user is advised to confirm results by another method.
3. The results are valid up to 15 minutes of development of red bands.

As such respective BAFRA field offices were advised to send the positive samples if any to National Food Testing Laboratory for further confirmation by using qPCR.

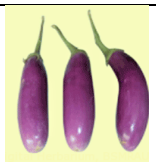
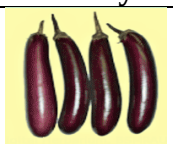
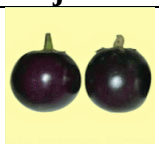
4. Result and discussion

A total of 280 samples were tested by 5 BAFRA offices as mentioned in table 4 below and the details of samples tested by respective BAFRA offices are provided in Table 5, 6, 7, 8, and 9 below:

Table 4: Total samples tested

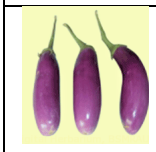
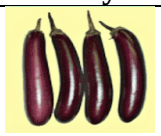
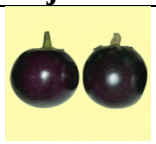
Sl.#	Varieties of Brinjal tested	Nos. of samples tested
1		89
2		89
3		52
4		50
TOTAL NUMBER OF SAMPLES TESTED		**280**

<u>Table 5: Test result from BAFRA Thimphu</u>

Sl.#	Source of Brinjal (Proper add if known)	Variety of Brinjal tested				Date of test
						
1	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17
2	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17
3	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17

4	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17
5	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17
6	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	13-11-17
7	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	07-12-17
8	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	07-12-17
9	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	07-12-17
10	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	07-12-17
11	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
12	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
13	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
14	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
15	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
16	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18

Table 6: Test result from BAFRA Samtse

Sl.#	**Source of Brinjal (Proper add if known)**	**Variety of Brinjal tested**				**Date of test**
						
1	Falakata & Dhupguri West Bengal	-ve	-ve	Not available on test date	-ve	11-12-17
2	Falakata & Dhupguri West Bengal	-ve	-ve	Not available on test date	-ve	22-12-17
3	Falakata & Dhupguri	-ve	-ve	Not	-ve	04-01-18

	West Bengal			available on test date		
4	Falakata & Dhupguri West Bengal	-ve	-ve	Not available on test date	-ve	24-01-18
5	Falakata & Dhupguri West Bengal	Not available on test date	-ve	-ve	-ve	02-02-18
6	Dhupguri, West Bengal	Not available on test date	-ve	-ve	Not available on test date	12-02-18
7	Dhupguri, West Bengal	-ve	-ve	-ve	-ve	20-02-18
8	Dhupguri & Falakata, West Bengal	-ve	-ve	-ve	-ve	07-03-18
9	Dhupguri & Falakata, West Bengal	-ve	-ve	-ve	-ve	09-03-18
10	Dhupguri & Falakata, West Bengal	-ve	-ve	-ve	-ve	15-03-18
11	Bihar, Dhupguri & Falakata West Bengal	-ve	-ve	Not available on test date	-ve	23-03-18
12	Dhupguri & Falakata West Bengal	-ve	-ve	Not available on test date	-ve	28-03-18
13	Dhupguri & Falakata, West Bengal	-ve	-ve	Not available on test date	-ve	06-04-18
14	Dhupguri & Falakata, West Bengal	-ve	-ve	Not available on test date	-ve	10-04-18

Table 7: Test result from BAFRA Phuntsholing

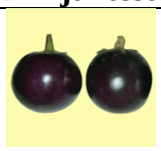
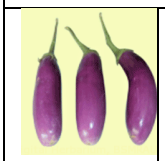
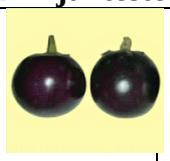
Sl.#	Source of Brinjal (Proper add if known)	Variety of Brinjal tested				Date of test
						
1	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	09-12-17
2	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	10-12-17
3	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	18-12-17
4	Jaigoan, India	-ve	-ve	-ve	Not available on test date	19-12-17
5	Jaigoan, West Bengal	-ve	-ve	-ve	Not available on test date	20-12-17
6	Jaigoan, West Bengal	-ve	-ve	-ve	Not available on test date	20-12-17
7	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	21-12-17
8	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	21-12-17
9	Jaigoan, West Bengal	-ve	-ve	-ve	Not available on test date	25-12-17
10	Jaigoan, West Bengal	-ve	-ve	-ve	-ve	27-12-17
11	Falakata, West Bengal	-ve	-ve	-ve	-ve	27-12-17
12	Falakata, West Bengal	-ve	-ve	-ve	-ve	29-12-17
13	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	15-02-18
14	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	26-02-18
15	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	2-03-18

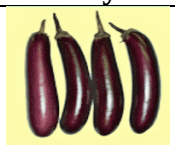
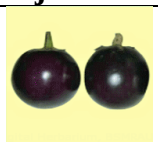
Table 8: Test result from BAFRA Gelephu

Sl. #	Source of Brinjal (Proper add if known)	Variety of Brinjal tested				Date of test
						
1	Falakata, West Bengal	-ve	-ve	Not available on test date	Not available on test date	17-4-18
2	Dadgari, Assam	-ve	Not available on test date	Not available on test date	-ve	17-4-18
3	Falakata, West Bengal	-ve	Not available on test date	Not available on test date	-ve	17-4-18
4	Dadgari, Assam	-ve	-ve	Not available on test date	Not available on test date	17-4-18
5	Dadgari, Assam	-ve	-ve	Not available on test date	Not available on test date	17-4-18
6	Falakata, West Bengal	-ve	Not available on test date	Not available on test date	Not available on test date	17-4-18
7	Falakata, West Bengal	-ve	-ve	Not available on test date	Not available on test date	18-4-18
8	Falakata, West Bengal	-ve	-ve	Not available on test date	Not available on test date	18-4-18
9	Kamakhyaguri, West Bengal	-ve	Not available on test date	Not available on test date	Not available on test date	18-4-18
10	Santipur, Assam	-ve	Not	Not	-ve	18-4-18

			available on test date	available on test date		
11	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	18-4-18
12	Falakata, West Bengal	-ve	-ve	Not available on test date	Not available on test date	18-4-18
13	Falakata, West Bengal	Not available on test date	-ve	-ve	Not available on test date	18-4-18
14	Dadgari, Assam	-ve	-ve	Not available on test date	Not available on test date	18-4-18
15	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	19-4-18
16	Falakata, West Bengal	-ve	-ve	Not available on test date	-ve	19-4-18
17	Khamakhaa Guri, West Bengal	-ve	-ve	Not available on test date	-ve	19-4-18
18	Falakata, West Bengal	-ve	Not available on test date	-ve	-ve	19-4-18
19	Dadgari, Assam	-ve	-ve	Not available on test date	Not available on test date	19-4-18
20	Dadgari, Assam	-ve	-ve	Not available on test date	Not available on test date	23-4-18
21	Falakata, West Bengal	-ve	-ve	-ve	Not available on test date	23-4-18

22	Falakata, West Bengal	-ve	-ve	Not available on test date	-ve	23-4-18

Table 9: Test result from BAFRA Samdrupjongkhar

Sl.#	Source of Brinjal (Proper add if known)	Variety of Brinjal tested				Date of test
						
1	Tamalpur and Kumarikata, Assam	-ve	-ve	-ve	Not available on test date	06-12-17
2	Tamulpur, Assam	-ve	-ve	Not available on test date	-ve	12-02-18
3	Parki, Assam	-ve	-ve	Not available on test date	-ve	06-03-18
4	Kumrikata Assam	-ve	-ve	-ve	-ve	13-03-18
5	Parki, Assam	-ve	-ve	Not available on test date	-ve	27-03-18
6	Garage, Assam	-ve	-ve	-ve	-ve	03-04-18
7	Parki, Assam	-ve	-ve	Not available on test date	-ve	14-04-18
8	Parki, Assam	-ve	-ve	Not available on test date	-ve	24-04-18
9	Falakata, West Bengal	-ve	-ve	Not available on test date	-ve	24-04-18
10	Parki, Assam	-ve	-ve	Sample not available.	-ve	01-05-18
11	Rekhey, Deothang	Sample not available	-ve	Sample not available.	-ve	01-05-18
12	Falakata, West Bengal	-ve	-ve	Sample not available.	-ve	01-05-18
13	Gawahati, Assam	Sample not available.	Sample not available.	-ve	-ve	09-5-18

14	Falakata, West Bengal	-ve	-ve	Sample not available.	-ve	09-5-18
15	Parki, Falakata, West Bengal	-ve	-ve	Sample not available.	-ve	09-5-18

The result of this pilot survey suggests that there is no evidence of presence of Bt-brinjal in the study area/site. Since we have chosen five major sites of study viz., Thimphu, Samtse, Samdrupjongkhar, Gelephu and Phuntsholing, it may not be a representative study for the whole of Country.

As stated in study conducted before, BAFRA offices are required to conduct regular surveillance using the rapid test kits in order to detect and prevent introduction of GMOs in the country as per the Biosafety legislation.

5. Conclusion

In conclusion this surveillance study conducted in four entry points and one major town suggested that there is no evidence of import or introduction of Bt-brinjal in the Country.